

AFAMRL-TR-80-112

ADA144982

citation



THE EFFECT OF DESIGNATED POLLUTANTS ON PLANT SPECIES

Fifth Annual Report

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THE REGENTS OF THE UNIVERSITY OF CALIFORNIA

UNIVERSITY OF CALIFORNIA, IRVINE

IRVINE, ORANGE COUNTY, CALIFORNIA 92664

JANUARY 1981

20060706081

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FOR THE COMMANDER



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Director
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REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER AFAMRL-TR-80-112	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) THE EFFECT OF DESIGNATED POLLUTANTS ON PLANT SPECIES: Fifth Annual Report		5. TYPE OF REPORT & PERIOD COVERED ANNUAL REPORT July 1, 1979 - June 30, 1980
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) A. L. Granett O. C. Taylor		8. CONTRACT OR GRANT NUMBER(s) F-33615-76C-5005
9. PERFORMING ORGANIZATION NAME AND ADDRESS Regents of the University of California University of California Riverside, California 92521		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 62202F 6302/04/17
11. CONTROLLING OFFICE NAME AND ADDRESS Air Force Aerospace Medical Research Laboratory Aerospace Medical Division, AF Systems Command Wright-Patterson Air Force Base, OH 45433		12. REPORT DATE January 1981
		13. NUMBER OF PAGES 69
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		15. SECURITY CLASS. (of this report) Unclassified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE
16. DISTRIBUTION STATEMENT (of this Report) Approved for public release; distribution unlimited		
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)		
18. SUPPLEMENTARY NOTES		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number)		
Acid mist	Developing seeds	HCl
Acid precipitation	Diurnal	HF
Acid rain	Exposure chambers	Hydrogen fluoride gas (HF)
Age of plants	Field studies	Missile exhaust products
Air pollution	Greenhouse	Phytotoxic responses
Crop yields	Hydrogen chloride gas (HCl)	Plant species
		Probit analysis
		Soil bioassay
		Sulfuric acid
		Space shuttle
		Vandenberg AFB
		Ozone
20. ABSTRACT (Continue on reverse side if necessary and identify by block number)		
<p>The phytotoxicity of short (20 minute) exposures of HCl and HF gases and of liquid HCl were investigated in a series of studies under controlled greenhouse conditions and in the field. Field plants exposed weekly to gaseous HCl were injured when young, but final plant heights, biomasses, and flower numbers were reduced only by repeated extraordinarily high doses. No decrease in numbers was found in microflora inhabiting field soil repeatedly exposed to high HCl doses. Five plant species exposed to gaseous HCl for 20 minutes were compared. Injury to cabbage head leaves did not occur on inside leaves. Dudleya, a succulent</p>		

plant native to Vandenberg AFB, was highly resistant. Plants exposed to HCl plus ozone sustained greater injury than those exposed to either pollutant separately. Distilled water vapor removed large quantities of HCl from polluted air and the resultant acid mist was less phytotoxic than the original gas. Acid precipitation based on HCl was less phytotoxic than the same concentration of H_2SO_4 . With sensitive bean plants, 0.1% HCl was injurious while 0.01% was not. Plant or tissue age or time of treatment did not significantly alter the amount of injury caused by acid sprays. Gaseous HF was more toxic than HCl gas. Of eight species exposed to HF gas for 20 minutes, beans were most sensitive and barley and dudleya were most tolerant. Exposure to HF gas reduced seed germination and seedling growth. Unexposed seeds on exposed soil and rinsed exposed seeds also were affected. Age influenced fluoride uptake and leaf sensitivity to short exposures of HF.

SUMMARY

Solid fuel, powering large rockets, produces huge clouds of hydrogen chloride (HCl) or hydrogen fluoride (HF) gases during launch. We studied the response of plants to short exposures of HCl or HF gases or to mist or rain containing HCl. Findings are as follows:

Field plants were exposed to six weekly 20-minute exposures of HCl.

- Measurements of plants from 10 weekly sacrificial harvests indicated that the highest gas concentration reduced height, dry weight, flower numbers, and bud production.
- Field soil exposed to weekly high HCl levels did not differ from unexposed control soil in numbers of bacteria or fungus populations recovered.

Five plant species, grown and exposed to single 20-minute exposures of HCl under greenhouse conditions, were compared within species and with other exposed plants.

- Dudleya, a succulent native of Vandenberg AFB was highly resistant.
- Cabbage heads exhibited injury only on exposed leaves; inner leaves were unaffected or incurred slight basal necrosis.
- These tests facilitated the compilation of a table comparing the resistance of 26 plant varieties to HCl gas.

Plants exposed to HCl for 20 minutes plus ozone for 90 minutes received more injury than when exposed to either pollutant alone. Injury was not related to whether the HCl exposure occurred at the beginning, middle, or end of the ozone episode.

Gas-laden ground clouds produced by rockets may encounter high levels of atmospheric water vapor resulting in an acid mist or acid rain that may impact vegetation. These conditions were simulated in the greenhouse to test plant response.

- Acid mist, created by sonicating distilled water into a chamber in which HCl was being generated, reduced the amount of detectable HCl as well as the amount of expected visible injury.
- Acid rain, produced by spraying leaves with aqueous HCl, caused injury on most exposed leaves at concentrations between 0.1 and 1.0% HCl (v/v).
- Citrus seedling leaf-drop occurred as spray concentrations increased.
- Bean and zinnia plants weighed one week after acid spray treatment showed weight reductions reflecting injury.

- Plant age was a factor in a plant's susceptibility to injury from HCl sprays.
- Plant response to sprays was not influenced by environmental factors of temperature, light, or relative humidity that changed over a diurnal period.
- HCl sprays caused less injury than treatments with the same concentrations of H₂SO₄.

Considerable literature details HF gas as a long-term, low-level toxicant with phytotoxic potential far exceeding HCl. Our findings concerned short, 20-minute exposures at elevated concentrations were:

- Seeds exposed to HF gas or seeds incubated on soil or filter paper exposed to HF gas had germination rates and subsequent seedling growth which was less than controls.
- Plants exposed to HF for 20 minutes showed species differences: dudleya was rarely injured while other plants were highly sensitive.
- Burning powdered leaf tissue in an oxygen atmosphere released fluorides for measurement with a specific-ion electrode.
- Plant age and gas concentration influenced HF effects: mature, developed bean primary leaves appeared more susceptible to visible injury and fluoride uptake than young or old leaves.

PREFACE

This is the fifth and final report of work performed by members of the Statewide Air Pollution Research Center, University of California, Riverside during the period from July 1, 1979 to June 30, 1980. The project was sponsored by Air Force Contract F-33615-76C-5005 to the University of California, Irvine.

The authors appreciate the cooperation and aid of Air Force contract monitor Lt. Col C. B. Harrah, Toxic Hazards Division, Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio. We also acknowledge the aid of E. A. Allingham, Dr. T. Bruhns, T. Carson, D. Duncan, A. L. Lee, M. R. Shulte, and C. L. Simpson during various portions of this project. Dr. Bruhns conducted most of the fluoride work; Mr. Schulte took the photographs and prepared many of the graphs. The advice and cooperation of Major W. Cairney, Ph.D., U. S. Air Force Academy, Colorado, during the soil studies was valuable.

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INTRODUCTION

This project concerns the effects of Air Force related pollutants on terrestrial vegetation. Support for these studies came from the Air Force as the Environmental Toxicology portion of a large program. Air Force interests and time restraints further limited this broad mandate to the effects of exhaust products of solid fuel booster rocket engines on plants found in the vicinity of Air Force space shuttle activities, Vandenberg Air Force Base, California. Studies during the past five years have dealt with the major exhaust constituents, hydrogen chloride (HCl) gas and aluminum oxide (Al_2O_3) particles (Granett and Taylor, 1976, 1977, 1978, 1979, 1980a, 1980b). Phytotoxicity of gaseous hydrogen fluoride (HF), the chief exhaust product of a more powerful rocket fuel that may find use in future Air Force operations, was investigated.

The unique nature of the source of the investigated toxicants separates these investigations from other botanical air pollution work. Rockets create large ground clouds which remain intact for a certain period. As the cloud drifts, it may impact terrestrial plants before dispersing. High pollutant concentrations and short (under 30 minutes) residence times restrict experimental procedures.

Earlier we reported that Al_2O_3 particles did not cause detectable plant injury and that injury caused by HCl plus Al_2O_3 was not greater than that caused by HCl alone (Granett and Taylor, 1978). Plant reactions to fumes created by burning pieces of solid fuel duplicated the bifacial necrosis and glazing seen after exposure to gaseous HCl.

Several avenues of investigation were followed during 1980. Sensitivities and yields of crops planted and exposed to gaseous HCl under field conditions were measured. The interacting influences of ozone and HCl on plants were studied in greenhouse chambers.

Moisture relationships with HCl were investigated by creating fog or mist in an exposure chamber simultaneously with HCl gas and by spraying plants with solutions of aqueous HCl.

The phytotoxicity of HF was investigated by comparing the sensitivity of various plant species and of plants of different ages. Seeds were incubated after being exposed to HF gas for short periods to determine whether germination rates and seedling development were altered.

MATERIALS AND METHODS

EXPOSURE EQUIPMENT

HCl and Ozone Chambers

A nine-chamber facility was developed to increase efficiency and flexibility in fumigation experiments. The chambers were standard continuous-stirred tank reactors (CSTR) design (Heck et al., 1978), each 1.37 m high by 1.37 m diameter (Figure 1). The units, constructed of wooden supports and a

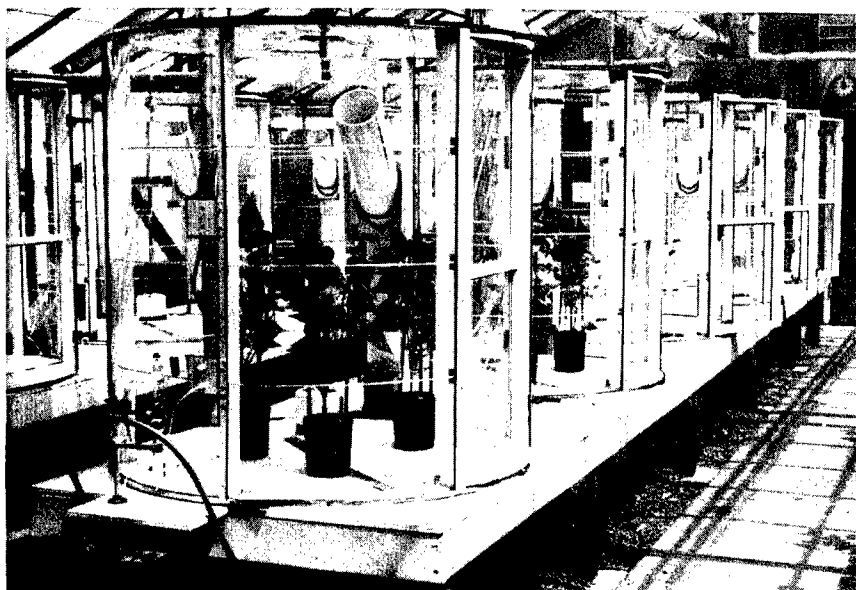


Figure 1. Bank of nine continuous-stirred tank reactor chambers used for exposing plants to gaseous HCl or ozone.

plywood base, had 5-mil Tedlar film covering all surfaces. An electric motor mounted above each chamber rotated four stirring paddles at ca 120 rpm. Greenhouse air that was charcoal filtered entered a 1.0 m long, 20 cm diameter PVC inlet tube entering the side of the chamber near the top. Pollutants and air mixed within the inlet tube and exited the chamber through a base-mounted, single 10 cm diameter outlet tube. A 23 cm diameter manifold connected all outlet tubes to a charcoal filter. A fan drew chamber exhaust through the filter, releasing cleansed air outside the greenhouse.

HCl, supplied as a 40% mixture of dry gas in nitrogen, entered a PVC manifold under low pressure. Needle valves and flowmeters on a control panel regulated gas flowing through 3 mm diameter Teflon tubing from the manifold to each chamber. A similar panel with a stainless steel manifold regulated the flow of ozone to each chamber. Any chamber could be supplied with HCl gas, ozone, or both pollutants.

Field Exposure Equipment

For field tests, pollutant was supplied to portable chambers constructed of a PVC tubing frame covered with Tedlar film (Granett and Taylor,

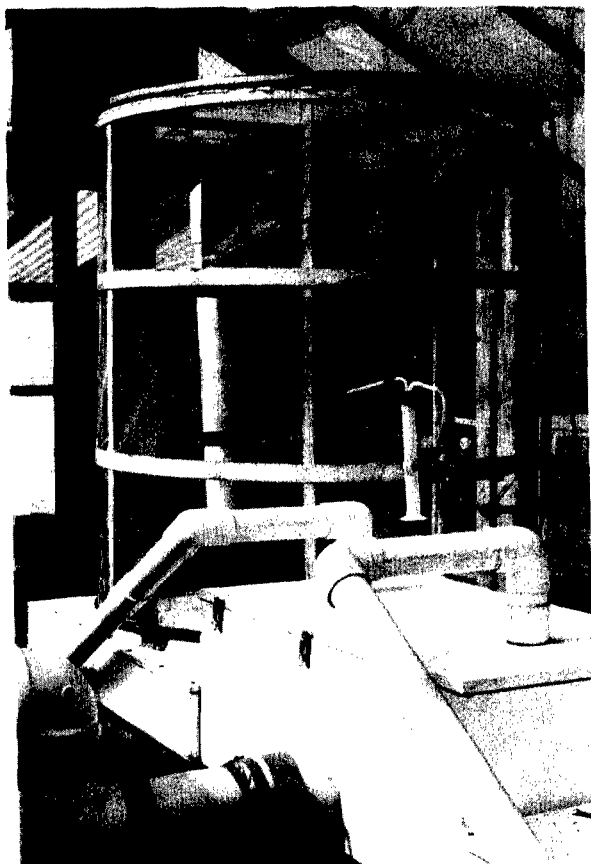


Figure 2. One of two continuous-stirred tank reactor chambers used for exposing plants to gaseous HF. Inlet and exhaust tubes and filter box are visible.

1979). A separate motor rotated stirring paddles within the chamber during exposures.

HF Chambers

HF exposure chambers consisted of two metal-framed CTSR units each 1.21 m high by 1.05 m diameter previously used by Granett and Taylor (1978) for HCl work (Figure 2). Inserted between the shared exhaust manifold and the exhaust fan was a 1.0 x 0.6 x 0.6 m sealed plywood filter box containing fluoride-adsorbing limestone ("crushed oyster shell") pellets in a plastic meshwork.

HF Generator

The HF generator consisted of Orion model 2 syringe pumps forcing aqueous hydrofluoric acid solution from a 20-ml plastic syringe into thin Teflon tubing. The tubing entered a large industrial oven (Blue M Electric Co., Blue Island, Illinois) bringing the acid to a Teflon T-union (Figure 3). Nitrogen carrier gas, heated to ca 100°C in 6-mm diameter copper tubing coiled in the oven, entered the T-union volatilizing the solution. HF gas was transported in a 4.6-m coil of 6-mm Teflon tubing inside the oven before traveling through insulated plastic tubes to the chamber intake manifolds. HF gas concentration was adjusted by changing solution concentration, syringe pump speed, carrier gas flow rate, or flow rate through the chamber. Condensation of hot HF gas was avoided.

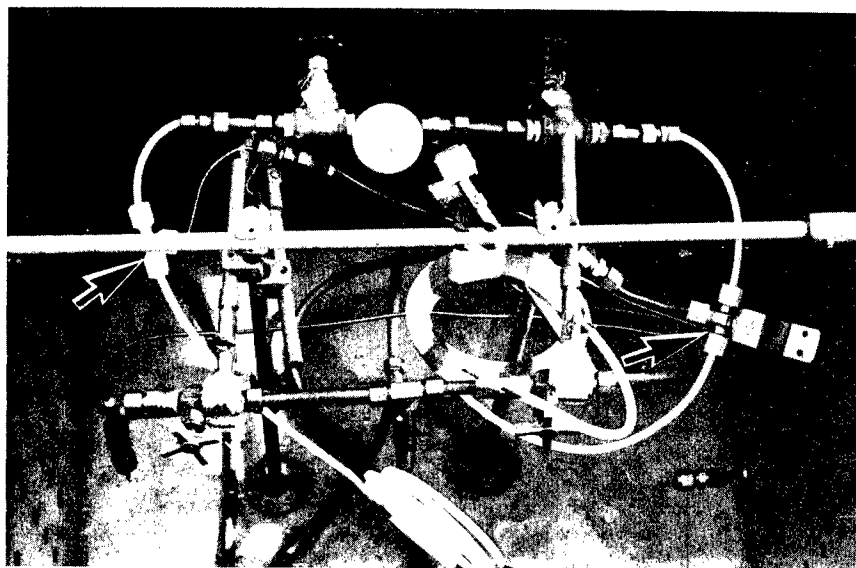


Figure 3. Interior of gaseous HF-generator oven showing T-unions (arrows), carrier gas tubes, and output coils.

PLANT PRODUCTION

Field Plants

Field preparation at the University plot in Riverside consisted of applying 89 kg nitrogen fertilizer and 12 kg pre-emergence weed killer (Dacthal) per hectare during plowing. Two-week-old zinnia and marigold plants, started in peat pots in the greenhouse, were transplanted to either side of 76 cm wide by 15 cm high beds. Plants were watered as needed, about weekly, using an irrigation trough running down the center of the beds. Orthene, a systemic insecticide, was applied for insect control.

Greenhouse Plants

Plants grown and exposed in a greenhouse at the Statewide Air Pollution Research Center were started from seed in 10-cm plastic pots or 350-cc Styrofoam cups filled with steam-sterilized U.C. Soil Mix II (Lerman, 1976). Plants were watered regularly and fertilized weekly with a nutrient solution (Hoagland and Aaron, 1950). Greenhouse conditions varied with daily and seasonal weather, but steam heat, whitewash paint, and evaporative coolers modified extremes in temperature, and charcoal filters eliminated most ambient atmospheric pollutants.

Seed Studies

Tomato (Lycopersicon esculentum Mill. var Ace) seeds were treated with pollutant gas then incubated in petri plates on moistened filter paper discs or on the surface of soil in Styrofoam cups. Seeds were incubated in the dark in a laboratory cabinet under reasonably constant temperatures (ca 21°C \pm 3°C).

EXPOSURES TO POLLUTANTS

Field Exposures of Plants to HCl

Portable chambers were placed over a double row of plants. Gaps along the edges were sealed with canvas aprons and soil. The stirring paddle motor was started and HCl was metered into an air stream produced by a squirrel fan (Granett and Taylor, 1979). Chambers were removed from the plants within 5 minutes after gas generation ceased.

Greenhouse Exposures of Plants to HCl, HF, and Ozone

HCl, HF, or ozone was brought to and maintained at desired concentrations in the exposure chamber prior to introducing test plants. Species were exposed for the desired period after which gas flow ceased and plants were removed.

Exposure of Plants to HCl Mist and Sprays

Acid mist conditions were achieved by introducing distilled water as an aerosol into a chamber in which HCl gas had reached a stable concentration. The water aerosol was generated with an ultrasonic nozzle (Sonicor Atomizer, Sonic Development Corp., Upper Saddle River, New Jersey) using nitrogen gas to create the needed pressure.

Acid sprays were administered to plants using plastic misting bottles to produce droplets. Acid solutions, prepared with concentrated HCl or sulfuric acid (H_2SO_4) and distilled water, were sprayed on plants until material dripped from leaves.

MEASUREMENTS

Injury

Plants were examined for effects of pollutant exposure about 24 hours after the episode. This allowed sufficient time for recovery from initial wilting and for the development of typical glazing, chlorosis, or necrosis. Leaf necrosis was the most common injury after exposure to HF gas, HCl gas or spray, or H_2SO_4 spray; abaxial glazing occurred when gaseous HCl pollutant stress was less severe. Visible injury was graded by noting type of injury and estimating percent of leaf area affected. Percent leaves injured and percent plants injured could be determined from the recorded data.

In some experiments, plants were harvested one week after exposure, oven-dried at 70°C for 24-72 hours, and weighed.

For the seed experiments, germination rate was determined and epicotyl and radicle lengths were measured.

Pollutant Concentrations

Concentration of HCl gas in the exposure chamber was determined by analyzing bubbler samples or by using a chemiluminescent Geomet model 401B HCl monitor (Granett and Taylor, 1979). For the nine-chamber facility, an arrangement was created using three wet test meters, three pumps, and three-way valves in the headhouse with plastic tubing to the bubblers located outside each greenhouse chamber such that any three atmospheres could be sampled for HCl simultaneously (Duncan and Granett, 1980).

Ozone concentrations were measured with a Dasibi model 4 monitor only when HCl was not present in a chamber. Teflon tubing from each chamber, a vacuum pump, valves and a manifold allowed the monitoring of any chamber from the headhouse.

HF gas was measured by bubbling 20 liters of atmosphere through an aqueous solution in a plastic graduated cylinder (Figure 4). The resulting solution was mixed with total ionic strength adjusting buffer (TISAB) and measured for fluoride ion with a specific ion electrode (Orion model 90-01) and an Orion model 901 ionanalyzer.

Aqueous solutions used for the acid spray experiments were made by diluting concentrated HCl and H₂SO₄ with distilled water.

HF Tissue Analysis

Leaves of bean plants exposed to HF were collected 48 hours after exposure. Some leaves were washed (Intersociety Committee on Methods for Ambient Air Sampling and Analysis, 1969) for 30 seconds in a polyethylene container in a solution of 0.05% Alconox and 0.05% tetrasodium salt of ethylene-diamine-tetraacetic acid (Na₄EDTA). Tissue was rinsed for 10 seconds in each of three beakers of deionized water. All leaves were dried at room temperature for 24 hours then at 75°C for 36 hours.

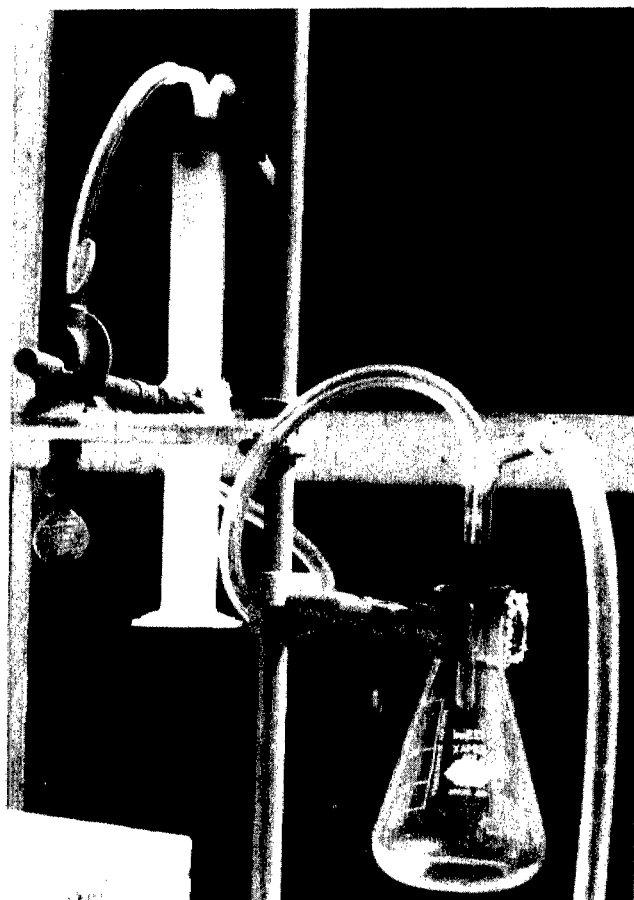


Figure 4. Plastic graduated cylinder used for fluoride-resistant bubbler for sampling gaseous HF.

Blade portion of leaves ground in a Wiley Mill passed through a 40-mesh screen. Replica samples weighing 0.05 g were mixed with 0.02 g sodium perchlorate, wrapped in ashless filter paper (Sample Wrappers, Cat. No. 6513-C75, A. H. Thomas Co., Philadelphia, Pennsylvania), and burned in an oxygen atmosphere (Levaggi et al., 1971). The released fluoride, absorbed in an alkaline solution, was measured with the specific ion electrode.

Soil Bioactivity

Samples from HCl-exposed soil were submitted to the U.S. Air Force Academy where the soil was diluted with water and plated on different media. After incubation, colonies of bacteria and fungi were counted.

Environmental Parameters

Temperature, relative humidity, light intensity, and photosynthetically active radiation (PAR) were measured as previously described (Granett and Taylor, 1979).

PHYTOTOXICITY OF GASEOUS HYDROGEN CHLORIDE

FIELD STUDIES

Earlier studies described field work with systems using either single pieces of solid rocket fuel or continuously flowing dry HCl gas (Granett and Taylor, 1979). Variability in plant sensitivity was indicated by the differences in visible injury from plant to plant as well as from species to species and from one location (Vandenberg Air Force Base) to another (Riverside, California). Growth, development, and yield effects were detailed in the study described below.

Effect on Yield

Zinnia and marigold plants transplanted to a Riverside field were exposed weekly for 6 successive weeks to one of four concentrations of HCl (Table 1). The exposures were replicated eight times in each of two blocks. One zinnia and one marigold plant per cell were sacrificed each week prior to exposing the remaining plants in that cell. Flowers were counted on harvested plant tops which were then dried, weighed, and measured.

Harvests continued 4 weeks after final exposure. Final harvest on week 10 revealed stunted plant development for those plants of either species exposed to highest HCl levels (110 mg m^{-3}). Zinnia bud and flower production after repeated HCl exposures was not greatly diminished, but marigold plants exposed to $110 \text{ mg HCl m}^{-3}$ had not yet flowered 4 weeks after exposures ceased. Greatly reduced seed numbers were recorded for zinnia plants exposed to the highest toxicant level (Table 2). Exposure effects were illustrated using the sacrificial and final harvest data to create growth rate curves (Figures 5-8). Both height and weight were significantly depressed in plants subjected to the highest gas concentrations compared to plants exposed to low levels or no HCl (Figures 5 and 6). Likewise, numbers of buds and flowers were greatly reduced for marigolds exposed to the $100 \text{ mg HCl m}^{-3}$ dose

TABLE 1
CONCENTRATIONS OF HCl GAS (in mg m⁻³) DURING WEEKLY 20-MINUTE FIELD EXPOSURES

Week	Gas Flow Setting			
	0	2	4	8
1	0	3.7	23.4	106.5
2	0	0.5	12.6	100.7
3	0	3.4	20.9	116.7
4	0	3.3	25.4	119.3
5	0	2.3	20.8	107.8
6	0	2.2	21.1	105.0
Average	0	2.6 ± 2.1	20.7 ± 5.6	109.5 ± 15.7

TABLE 2
MEAN NUMBER OF SEEDS FROM FIELD PLANTS EXPOSED WEEKLY TO HCl GAS FOR SIX WEEKS AND HARVESTED FOUR WEEKS AFTER LAST EXPOSURE

HCl Concentration (mg m ³)	Marigold	Zinnia
0	66.0 ± 35.2 a ¹	17.0 ± 13.5 bc
3	51.8 ± 42.8 a	21.4 ± 18.9 ab
21	66.8 ± 30.0 a	30.6 ± 24.0 a
110	0.0 ± 0.0 b	5.1 ± 6.1 c

¹Means followed by the same letter are not significantly different at 5% level by Duncan's Multiple Range Test.

(Figures 7 and 8). A dose of 21 mg HCl m⁻³ for 20 minutes was near the injury threshold for these species when they were grown and exposed under greenhouse conditions. Both field hardiness and increased tolerance with age allowed repeated exposures at this potentially damaging concentration (21 mg m⁻³) with minimal effects on final plant yield. Pollutant at the largest concentration caused moderate injury to field plants, but would have severely injured greenhouse-grown plants. Response in field plants was also noticed at this elevated concentration as reduced biomass and height and decreased seed production. Moderate doses of HCl (20 mg m⁻³ for 20 minutes) may not significantly affect plant growth or yield in the field, but large doses (around 100 mg m⁻³) will.

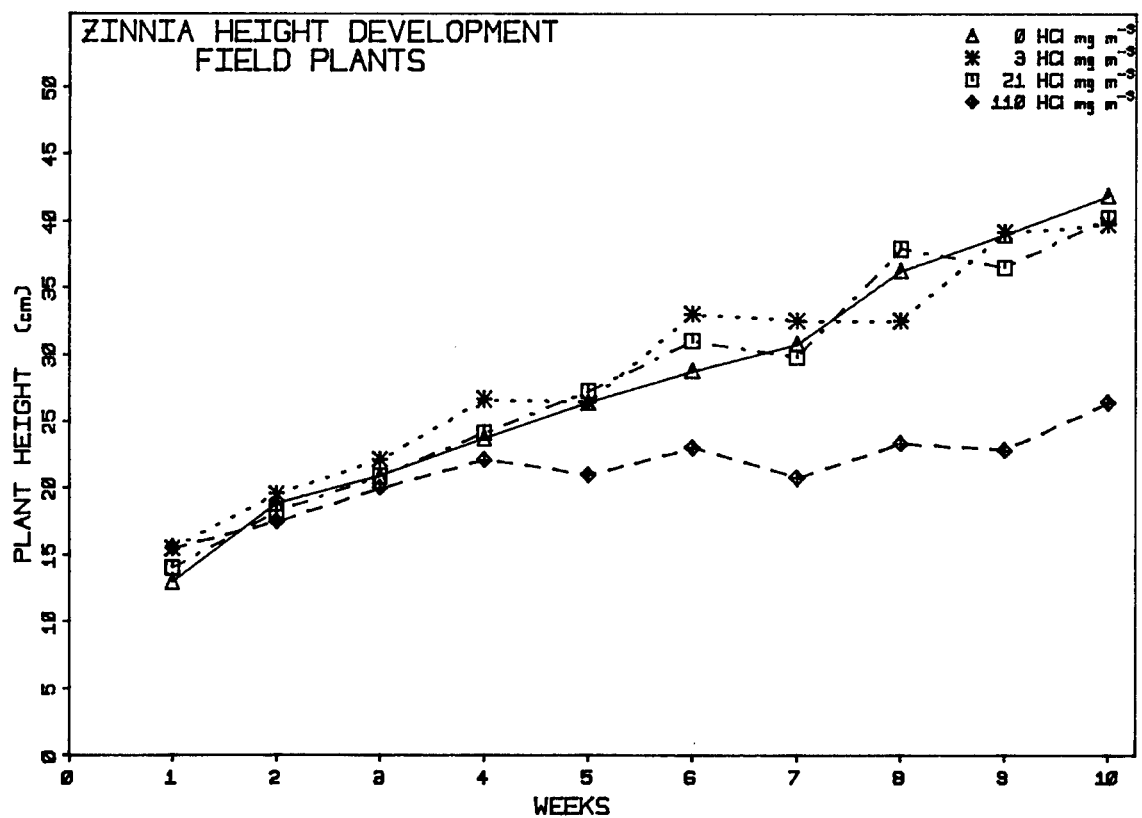
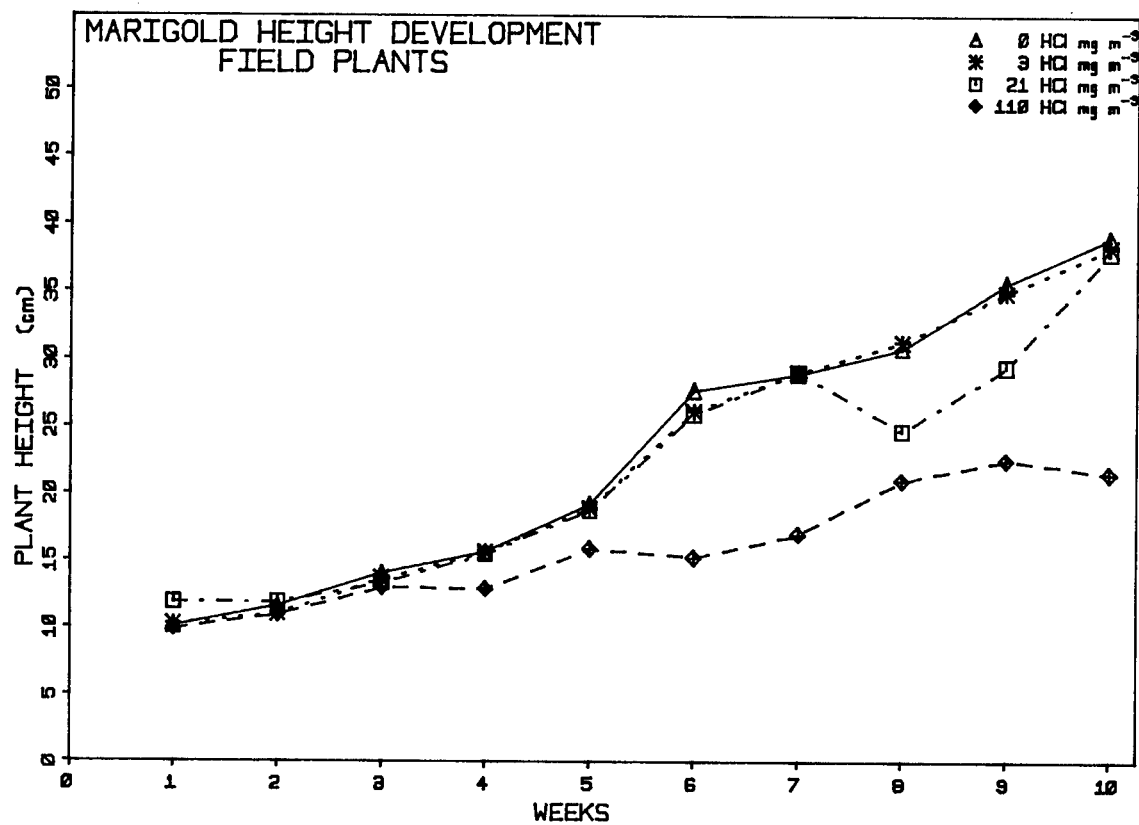


Figure 5. Height of field plants exposed weekly to 20-minute episodes of HCl gas. Top: marigolds; bottom: zinnias.

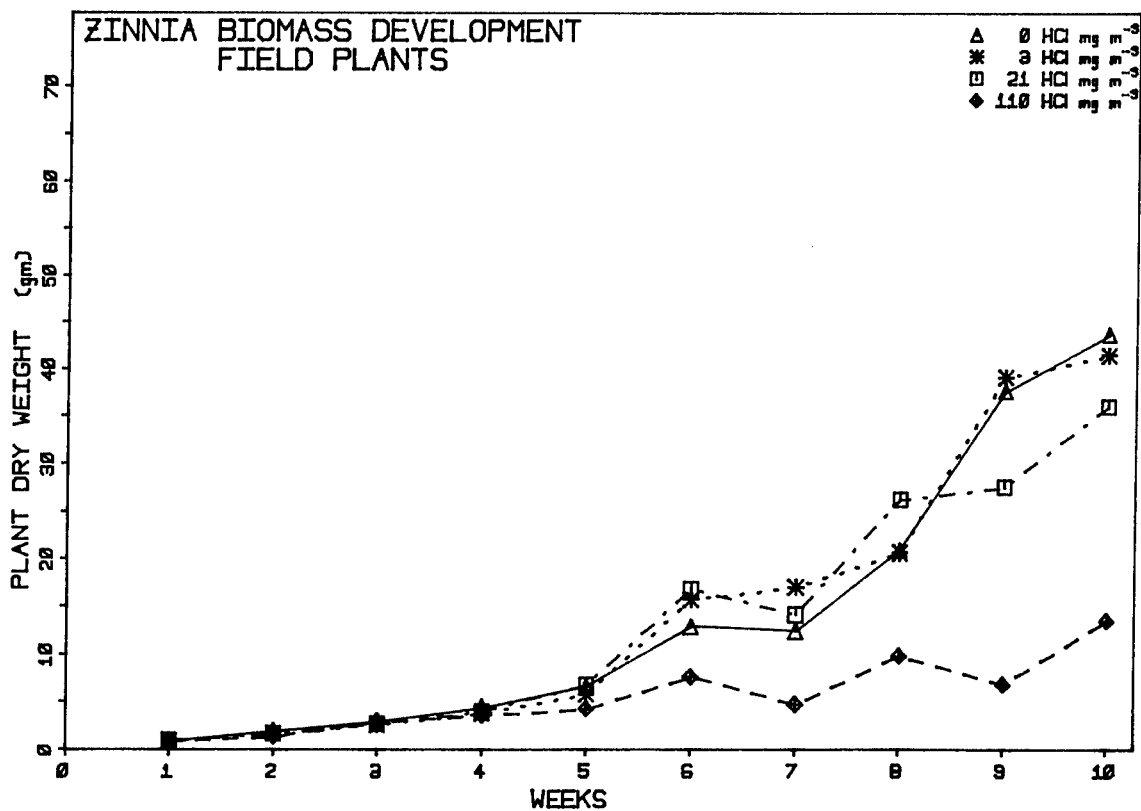
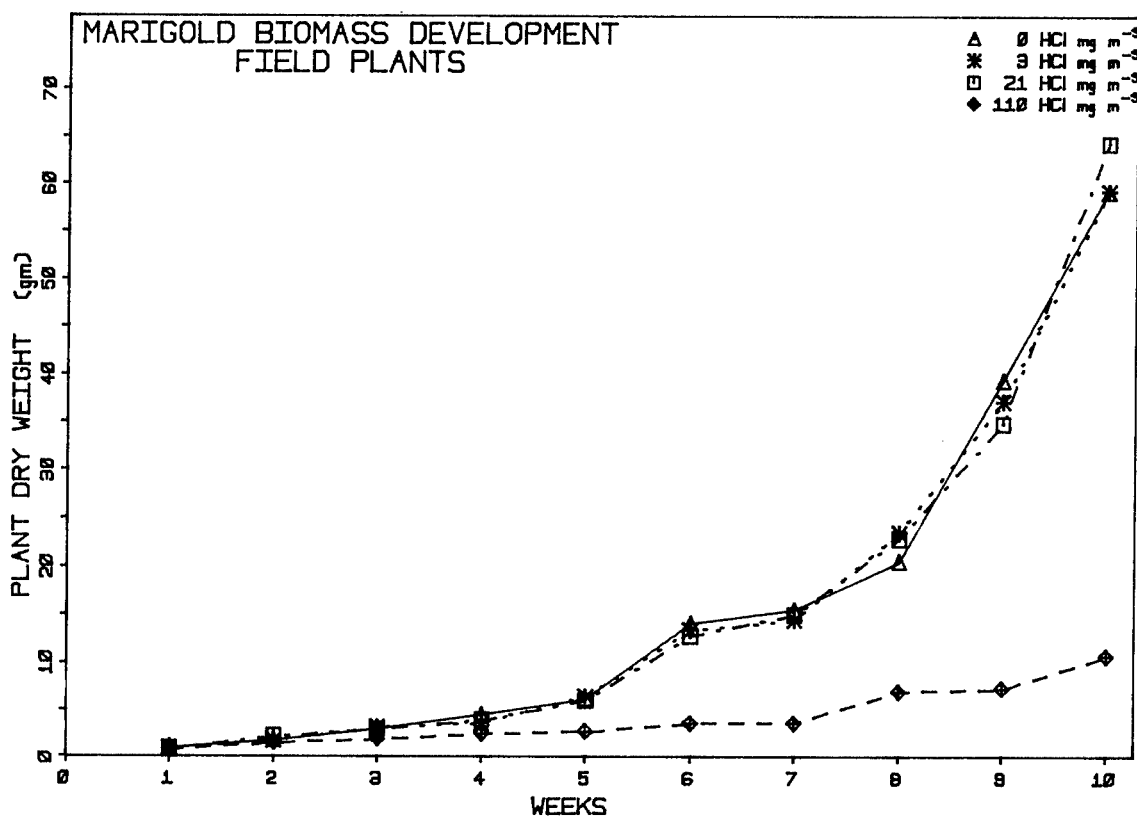


Figure 6. Biomass (dry weight) of field plants exposed weekly to 20-minute episodes of HCl gas. Top: marigolds; bottom: zinnias.

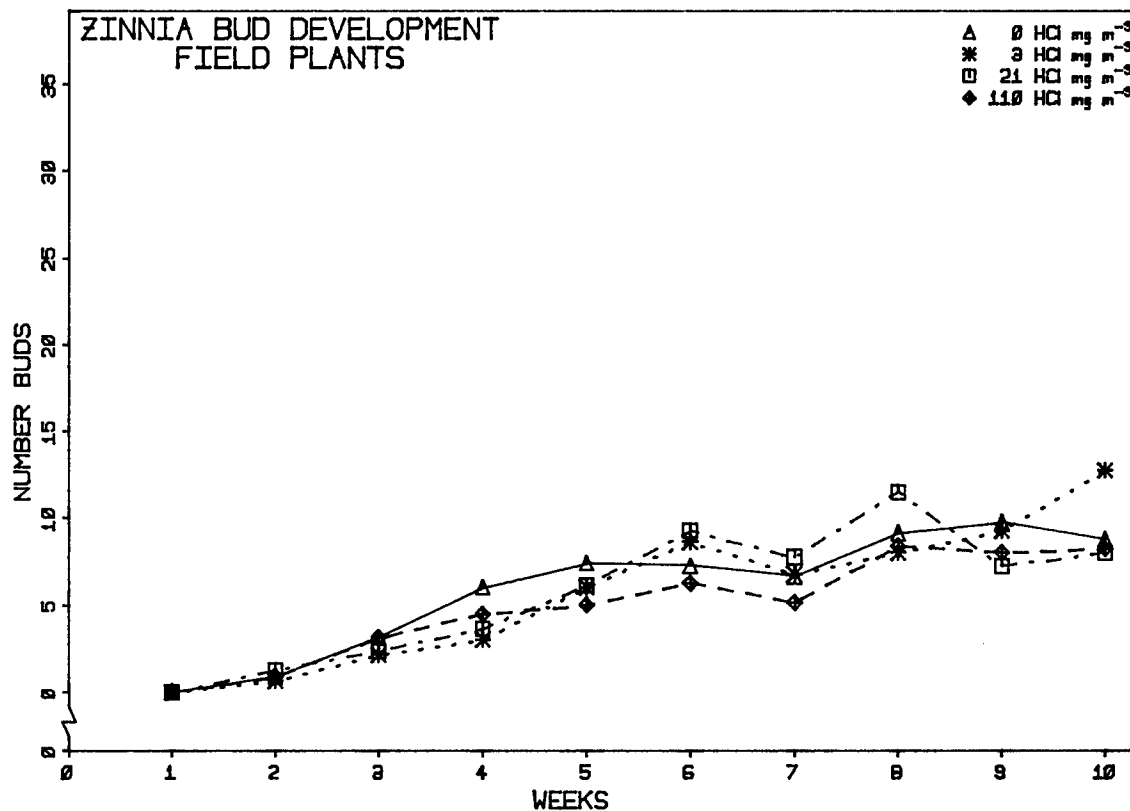
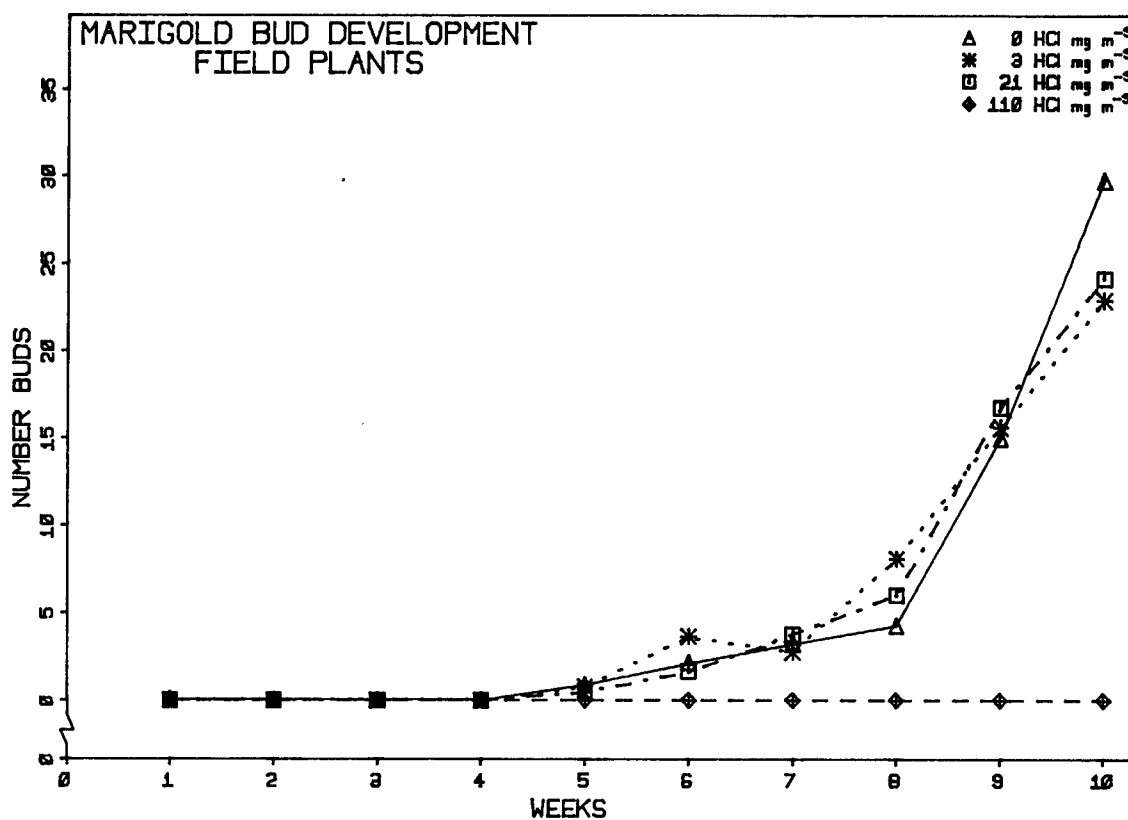


Figure 7. Number of flower buds on plants exposed weekly to 20-minute episodes of HCl gas. Top: marigolds; bottom: zinnias.

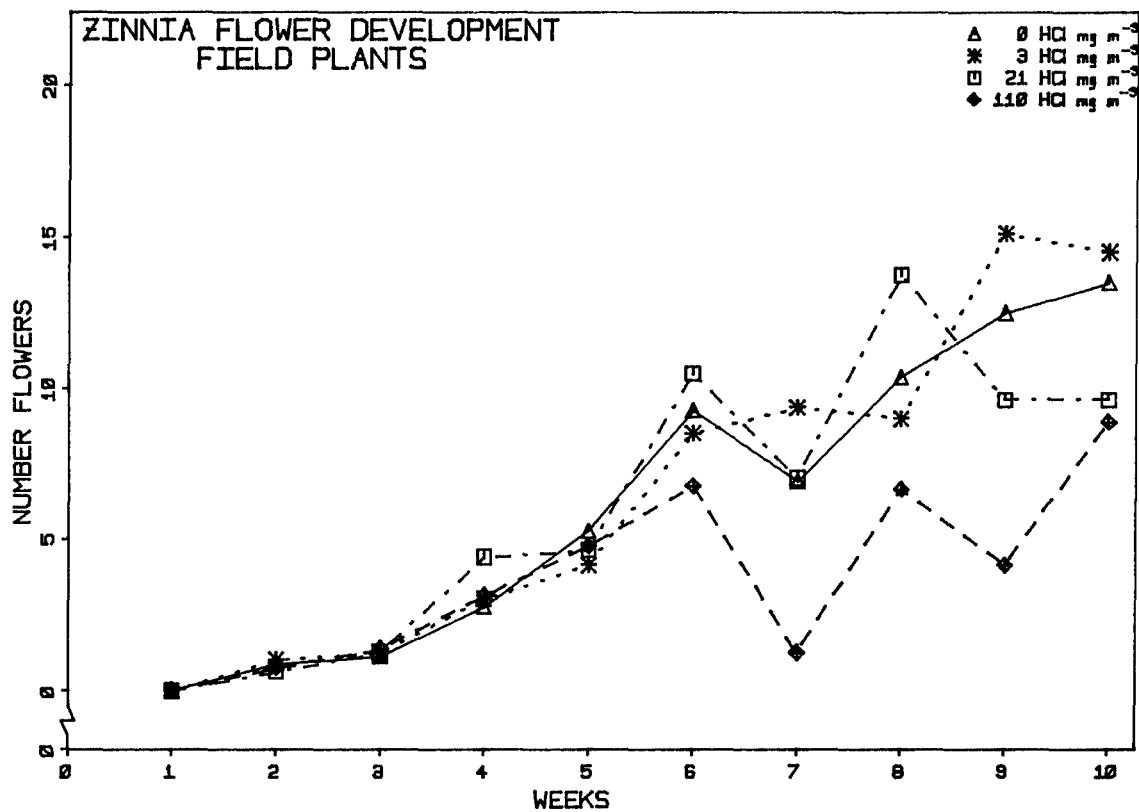
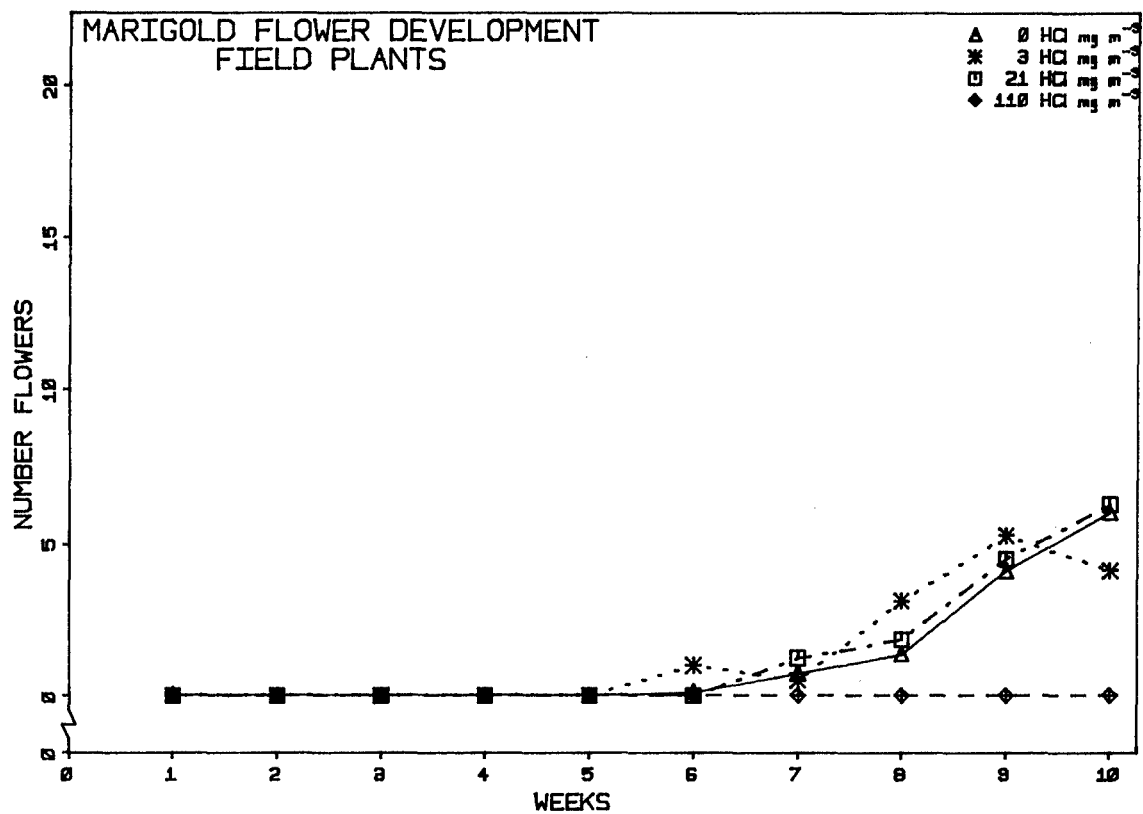


Figure 8. Number of flowers on plants exposed weekly to 20-minute episodes of HCl gas. Top: marigolds; bottom: zinnias.

Field Soil Bioassay

Following the field experiment described above, soil was sampled from areas exposed to the highest gas concentration (six weekly 20-minute doses of ca 110 mg HCl m⁻³ each) and from pollutant-free control cells. Samples came from the first cm of soil and were closer to ("wet") or farther away from ("dry") the irrigation trough. No attempt was made to determine soil moisture content. Each sample was thoroughly mixed and 1.0 g of a given sample was added to 99 ml sterile distilled water. A magnetic stirrer agitated the suspension for 5 minutes. The suspension, after settling 1 minute, was pipetted in 1-ml aliquots into 99 ml sterile distilled water. The new suspension was agitated 1 minute and further diluted to produce a final series of 10⁻², 10⁻⁴, 10⁻⁶, and 10⁻⁸. Aliquots were dispersed onto three media in sterile Petri plates with four replications per dilution for each sample. Plated samples were incubated at room temperature (24°C).

Three media were used to determine microorganism levels. Potato dextrose agar (PDA) at pH 5.6 was used for development of soil fungi. Mycophil agar (MA) at pH 4.7 was used to cross-check numbers and species determined by growth on PDA. Nutrient agar (NA) at pH 6.8 encouraged growth of bacteria. Cross-contamination was not a problem so bacterial and fungal inhibitors were not necessary.

NA plates were examined for bacteria 2 days after inoculation; no new colonies appeared after this time. PDA and MA plates were examined for fungi after 5 days. Colony counts on each plate were summarized as mean microflora values (Table 3). No statistical differences were found when the first set of means was analyzed. The second data-set, however, had significantly more bacterial than fungal colonies. Neither soil condition nor HCl treatment affected the number of microorganisms detected.

TABLE 3

MICROFLORA (X 10⁶ colonies) RECOVERED FROM SOIL SAMPLED FROM WETTER AND DRIER PORTIONS OF FIELD PLOTS EXPOSED WEEKLY TO HCl GAS

HCl Concentration (mg m ⁻³)	Bacteria		Fungi	
	Wet	Dry	Wet	Dry
First Data Set				
110	4.33 ¹	5.33	6.60	5.57
0	3.00	4.00	2.40	1.40
Second Data Set				
110	7.25	6.00	0.62	1.18
0	20.80	7.00	0.66	1.07
¹ \bar{x} for 4 replicate plates per sample				

SPECIES SENSITIVITY

Sensitivities of plant species to HCl gas have been reported earlier (Granett and Taylor, 1976, 1977, 1978, 1979). Additional species have been tested and their reaction to short periods of HCl gas considered. Mature plants of three species were exposed: cabbage (*Brassica oleracea* var. *capitata* L. c.v. Danish roundhead), California poppy (*Eschscholtzia californica* Cham.), and dudleya (*Dudleya caespitosa* (Haw.) Britt. & Rose). Preliminary trials indicated a gradient of 100 to 350 mg HCl m⁻³ would be necessary to induce injury. Exposures were conducted simultaneously in six chambers, each containing a different level of gas. All plants were exposed at the same time of day and under the same environmental conditions.

Cabbage

Plants were 28 weeks old with full heads (ca 15 cm diameter) when exposed. A day after the 20-minute exposures, the outer leaves from each head were peeled off and graded for injury. Glazing and necrosis occurred on parts of leaves exposed to the atmosphere. Injury on protected inner leaves was limited to basal glazing. Injury was dependent on gas concentration; no visible injury occurred on more than the first 14 leaves on any head (Table 4). The outer second or third leaf was usually most susceptible to HCl.

TABLE 4
CABBAGE LEAF INJURY AS A FUNCTION OF DEPTH INTO HEAD AND HCl CONCENTRATION

Leaf No. ¹	HCl Concentration (mg m ⁻³)					
	97	156	227	234	312	344
1	4 ²	19	29	42	58	42
2	0	29	33	50	58	42
3	4	29	42	25	54	50
4	4	25	29	25	54	38
5	6	17	25	23	42	38
6	4	10	12	15	33	29
7	4	8	19	15	19	19
8	0	8	6	10	6	17
9	0	4	2	6	4	15
10	0	2	0	2	2	6
11	0	0	0	2	2	8
12	0	0	0	4	0	8
13	0	0	0	0	0	4
14	0	0	0	0	0	4
15	0	0	0	0	0	0
Mean	1.5	8.9	11.4	12.4	19.6	18.8

¹Leaves numbered from outside head (#1) inward

² \bar{x} percent leaf area injured on leaves from 3 plants

Although injury was variable from leaf to leaf and plant to plant, average leaf area injured of the first 15 leaves was highly correlated ($r = 0.91$, $F = 83.7$, $p = 1\%$) with gas concentration (Figure 9).

Poppy

California poppy, indigenous to the Vandenberg Air Force Base area, blooms in the spring. Since flowering of individual plants could not be closely controlled under greenhouse conditions, data on flower sensitivity was limited. Floral injury did not occur following exposure to 75 mg HCl m^{-3} for 20 minutes.

Poppy plants trimmed of flowers, buds, and senescent leaves were 27 weeks old when exposed to the HCl gradient. Foliar injury ranged from margin necrosis to complete burning of the small, narrow, deeply lobed leaves. High concentrations also killed stem tissue. Grading consisted of counting number of leaves injured per plant (Table 5). Poppy plants were more susceptible than cabbage to injury from HCl; however, relatively high gas doses were needed to produce serious visible damage. No significant differences between means were found by analysis at the 5% level.

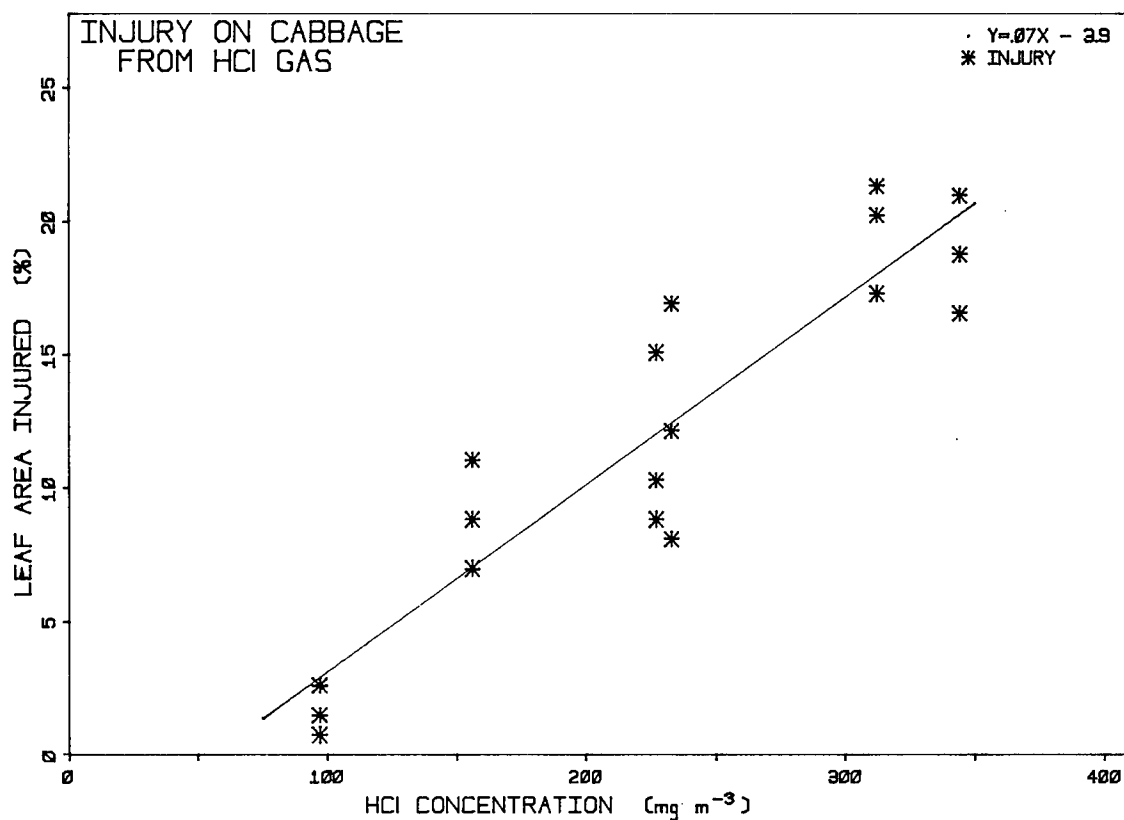


Figure 9. Leaf injury on cabbage plants exposed to HCl gas for 20 minutes. Each point is the average injury on 17 leaves of a single plant.

TABLE 5
LEAF INJURY (%) ON MATURE CALIFORNIA POPPY PLANTS EXPOSED TO
HCl GAS FOR 20 MINUTES

HCl Concentration (mg m ⁻³)	No. Leaves Injured (%)
97	62
156	62
227	86
234	83
312	99
344	100

Dudleya

Dudleya is a perennial herb with thick fleshy leaves. Experimental plants transferred from Vandenberg AFB to Riverside were vegetatively propagated over a period of 8 to 10 months and grew well under greenhouse conditions. Injury, in the form of small necrotic flecks resembling ozone response, followed exposure to high concentrations of HCl. Since more than half the exposed leaves were not injured by doses over 300 mg HCl m⁻³, extremely high HCl concentrations would be necessary to significantly harm this species (Table 6). Because greenhouse plants are often more sensitive than field-grown plants, even higher gas concentrations would most likely be needed to damage dudleya growing at the Base.

TABLE 6
LEAF INJURY ON MATURE DUDLEYA PLANTS EXPOSED TO HCl GAS FOR 20 MINUTES

HCl Concentration (mg m ⁻³)	No. Leaves Injured (%)	Leaf Area Injured (%)
97	5.8 ± 5.7 ¹ c	1.2 ± 1.05 b
156	1.6 ± 1.6 c	0.2 ± 0.2 c
227	20.9 ± 4.0 c	6.8 ± 5.2 ab
234	9.5 ± 6.7 b	1.2 ± 0.8 b
312	18.9 ± 9.4 b	3.3 ± 1.6 b
344	42.8 ± 3.9 a	10.4 ± 0.6 a

¹Means followed by the same letter are not significantly different at 5% level by Duncan's Multiple Range Test.

TABLE 7
SUDAN GRASS INJURY (% LEAF AREA) AS A FUNCTION OF
LEAF AGE AND HCl CONCENTRATION

Leaf No.	HCl Concentration (mg m ⁻³)							\bar{x}
	27.0	28.0	34.5	36.5	40.9	41.6	42.2	
1 ¹	0.0 ²	0.0	7.1	7.1	7.1	19.6	9.8	7.2 ³ y
2	8.9	12.5	23.2	37.5	35.7	24.1	33.9	25.1 x
3	17.9	16.1	33.4	42.9	39.3	33.9	41.1	32.1 w
4	13.4	6.2	17.9	17.9	17.9	14.6	27.1	16.4 z
\bar{x}	10.1d	8.7d	20.4d	26.4ab	25.0ac	23.0c	28.0ab	

¹Oldest leaf is #1

²Mean of leaf area injured for 7 plants

³Averages followed by same letters are not significantly different at 5% level by Duncan's Multiple Range Test

Sudan Grass

Sudan grass, Sorghum vulgare var. sudanese (Piper) Hitchc., was exposed to HCl gas when plants were 27 days old and had 4 leaves. Bifacial necrosis beginning mid-leaf extended toward the outside areas of exposed leaves. Plants were moderately sensitive to HCl with the third leaf more sensitive to injury than the other three (Table 7). Numbers of leaves with symptoms was a satisfactory measure of injury (Figure 10). The linear regression line, Injury (% leaves) = 1.9 [HCl] + 12.7, was statistically significant ($r = 0.91$, $F = 19.96$, $p = 1\%$) for the summary data based on the average injury on 7 plants. Leaf area injury also determined a significant ($r = 0.55$, $F = 20.15$, $p = 0.5\%$) linear regression line, Injury (% area) = 1.2 [HCl] - 21.0.

Pinto Bean

Beans, particularly Phaseolus vulgaris L. c.v. Pinto, have long been standard test plants for visible injury from air pollution (e.g., Feder and Manning, 1979). Pinto beans were relatively sensitive to short exposures of HCl gas but were somewhat unpredictable in their response. A steep reaction gradient existed where a small change in pollutant concentration produced a dramatic increase in injury. Thus injury threshold doses could not be predicted easily. To determine more accurately the threshold concentration necessary to produce visible injury on beans, plants were exposed for 20 minutes in a series of fumigations. The linear correlation of gas concentration and injury was significant for leaf area injured ($r = 0.59$, $F = 7.64$, $p = 5\%$) but not stastically significant (N.S.) for number leaves injured ($r = 0.337$, $F = 1.80$, N.S.) (Figure 11). When exposure time was reduced to 15 minutes, the

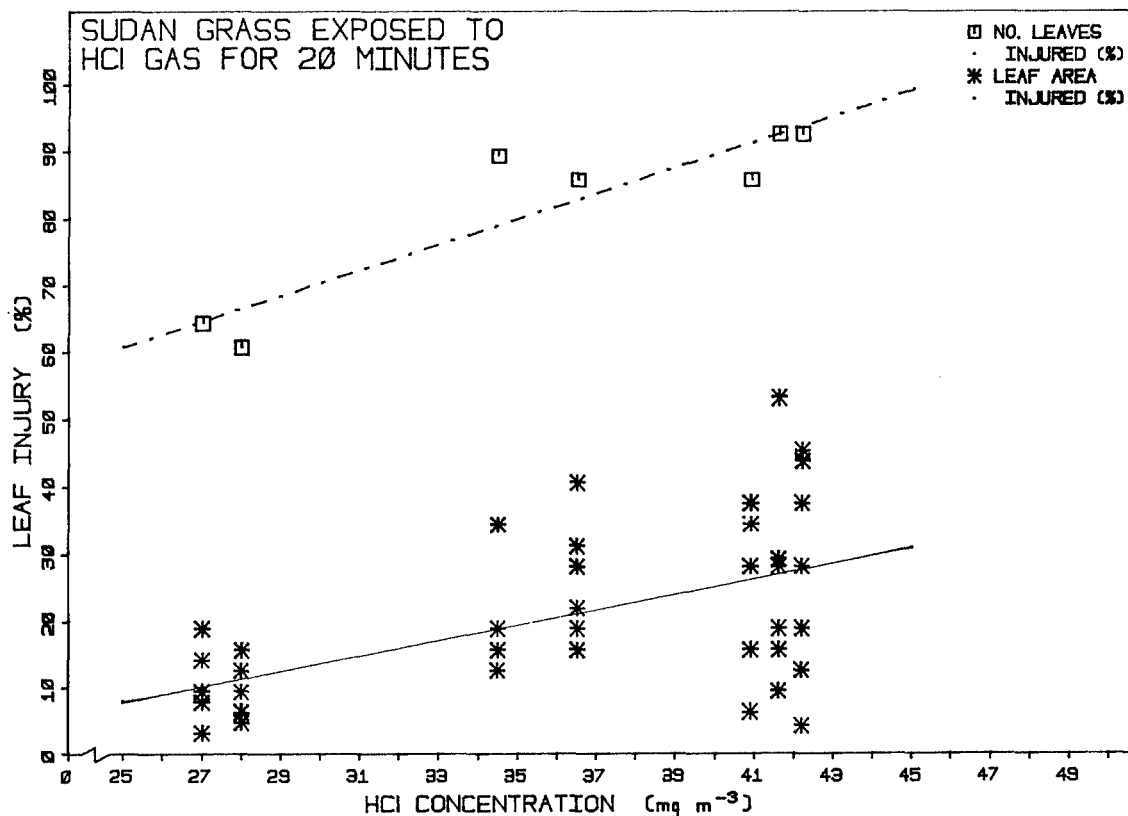


Figure 10. Sudan grass injury after exposure to HCl gas for 20 minutes. Leaf number data are seven-plant averages; leaf area data are for each plant exposed.

linear correlations of injury and concentration improved ($r = 0.72$, $F = 8.55$, $p = 5\%$ for leaf area injured and $r = 0.84$, $F = 19.98$, $p = 0.5\%$ for number leaves injured). Using a narrower range of concentrations for the 15-minute exposures improved the linearity of the response.

Measuring visible injury on pinto bean leaves has limited usefulness as a bioindicator of high levels of HCl in the atmosphere for short time periods. Perhaps combining visible reactions with some physiologic response, such as chloride uptake (Endress, Kitasako, and Taylor, 1979a), would be advantageous.

Probit Analysis Comparisons of Visible Injury Response

Previous reports (Granett and Taylor, 1978, 1979) discussed the use of probit analysis (Finney, 1971) for describing the reaction of plant species to HCl gas. Probit lines were constructed for the visible injury response of cabbage, dudleya, sudan grass, and pinto beans (15-minute exposure only) to HCl gas (Figure 12).

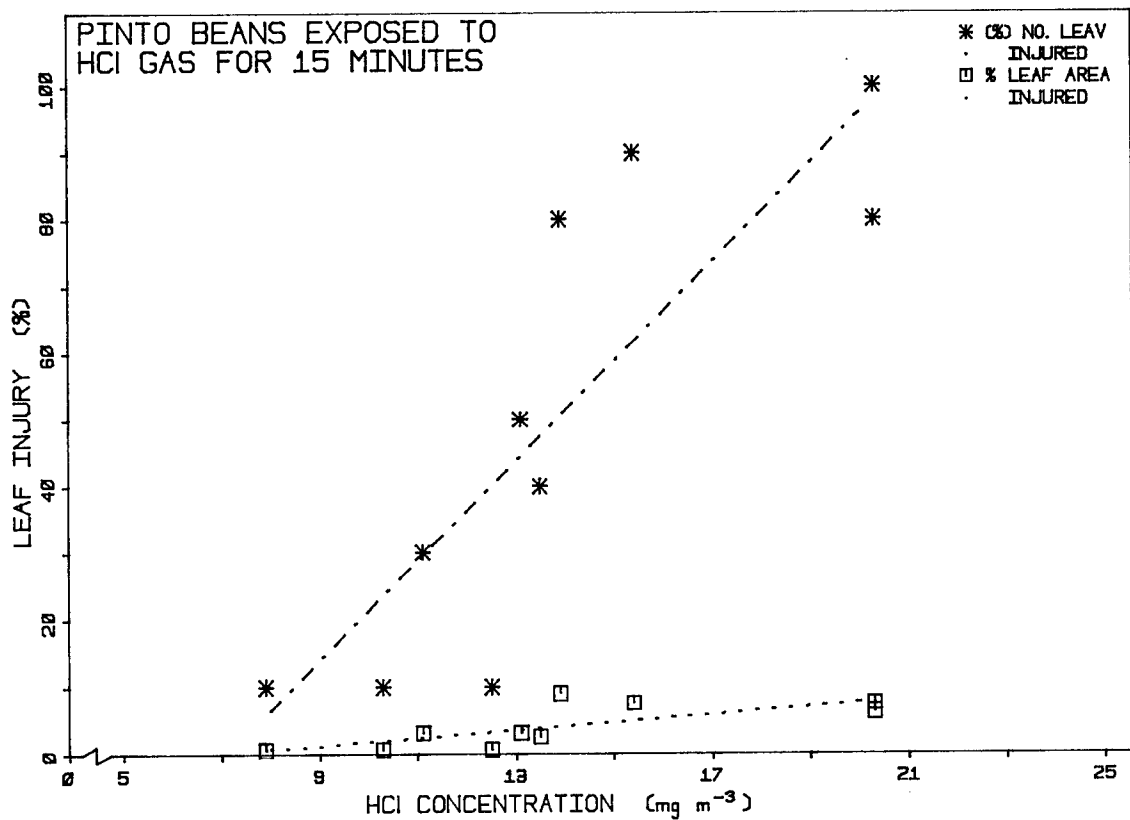
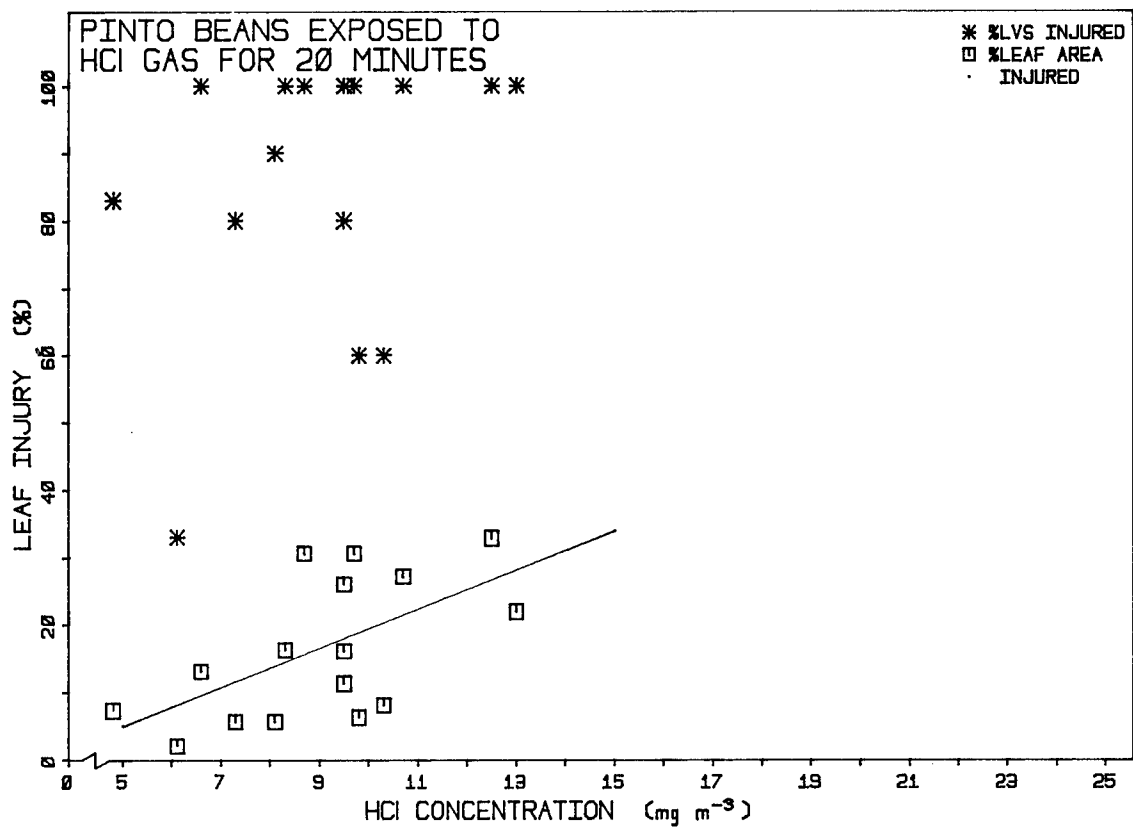


Figure 11. Leaf injury, expressed as number of leaves and leaf area injured, on bean plants exposed to HCl gas. Lines are linear regressions. Top graph: 20-minute exposure; bottom graph: 15-minute exposure.

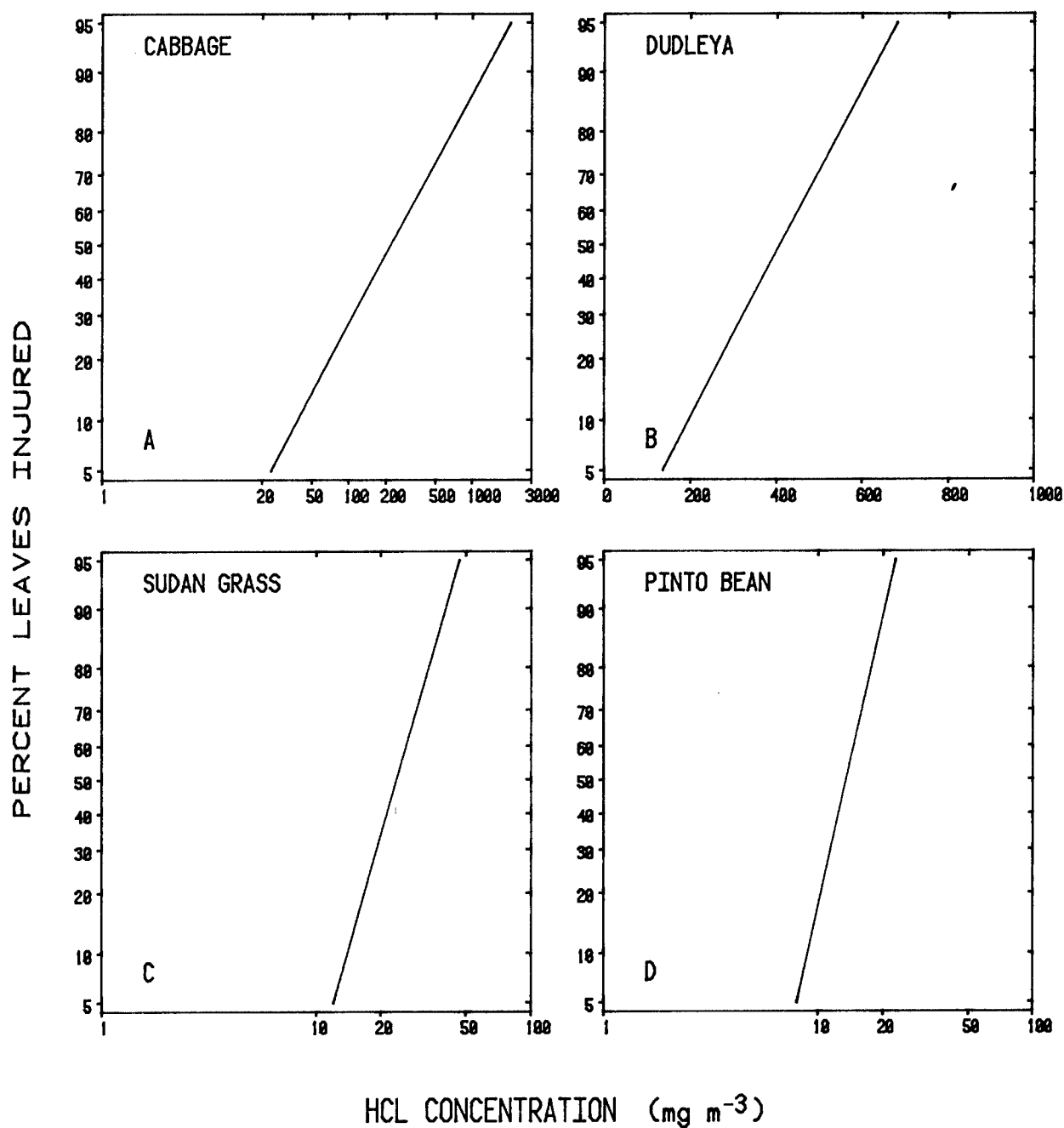


Figure 12. Probit analysis lines for (A) cabbage, (B) dudleya, (C) sudan grass and (D) pinto bean (15-minute) exposed to HCl gas. Probit percent is the injury (in % number of leaves injured) expected if plants were exposed to HCl at the corresponding concentration.

Based on these and earlier data (Granett and Taylor, 1978), certain groupings of tested species can be made indicating relative susceptibility of plants to HCl under greenhouse conditions (Table 8). Fast-growing, herbaceous species are more sensitive to injury than slower-growing, woodier species.

EFFECTS OF HCl GAS PLUS OZONE ON PLANTS

Photochemical smog, consisting chiefly of ozone (O_3), is a common urban air pollutant. Since smog can travel considerable distances from origins before dissipating, large HCl releases might occur concurrently with high O_3 levels.

Pinto bean and zinnia plants were fumigated with O_3 for 90 minutes. To test the phytotoxicity of two pollutants together, gaseous HCl was introduced for 20 minutes during the O_3 exposure. Episodes of five HCl gas concentrations took place at the start, middle, or end of the 90-minute periods. Treatments were repeated twice a day on three different days. Fumigations with 50 pphm O_3 were made on the first day and 35 pphm O_3 was used on the second and third days. Visible foliar injury was estimated 24 hours after exposure (Table 9) and after two weeks plant tops were harvested, dried, and weighed (Table 10).

Regression analysis and analysis of variance were used to test response means. Bean plant reactions to the pollutant stresses were usually significantly different from the zinnia reactions (Table 11). Exposing plants to either HCl or O_3 alone produced responses which increased in severity as gas concentrations increased (Table 12). All linear correlation coefficients were significant and were positive for injury and negative for dry weights (Table 13).

Reactions of plants exposed to HCl plus O_3 , were more severe at low HCl concentrations; but this relationship was unpredictable at higher HCl levels for both O_3 concentrations (Table 12). When analysis of variance was used neither the HCl concentration nor the time the HCl episode occurred within the O_3 fumigation were consistently significant variables (Table 14). The effect of HCl plus O_3 on the plants does not appear additive, nor were the reactions consistently synergistic or antagonistic. Environmental and other factors not monitored or analyzed in these studies may have played important roles in plant response.

PHYTOTOXICITY OF HYDROCHLORIC ACID

BACKGROUND

Precipitation containing acid has received considerable public attention (Graves, 1980). Rain and snow of low pH was first reported in Europe over 20 years ago and is now considered a serious problem in the Eastern United States with recent occurrences in the West. Sulfur dioxide and the nitric oxides create most "acid rain" as these industrial air pollutants combine with water vapor to form sulfuric (H_2SO_4) and nitric (HNO_3) acids, respectively.

TABLE 8

RELATIVE SENSITIVITIES OF GREENHOUSE CULTIVATED PLANTS TO HCl GAS

Plant	Scientific name	Variety/cultivar	Age (Weeks)
VERY SENSITIVE ¹ [80-100% injury at 25 mg m ⁻³]			
Bean	<u>Phaseolus vulgaris</u> L.	Pinto (V.I.III)	2
Marigold	<u>Tagetes patula</u> L.	Goldie	3
SENSITIVE [50-75% injury at 25 mg m ⁻³]			
Radish	<u>Raphanus sativus</u> L.	Comet	2
Sudan grass	<u>Sorghum vulgare</u> (Piper) Hitchc.	<u>Sudanese</u>	4
Tobacco	<u>Nicotiana tobacum</u> L.	Bell W-3	10
Zinnia	<u>Zinnia elegans</u> Jacq.	Cherry Gem	3
MODERATELY [50-100% injury at 50 mg m ⁻³]			
TOLERANT			
Aster	<u>Callistephus chinensis</u> (L.) Nees	Early bird white	7
Calendula	<u>Calendula officinales</u> L.	Flame beauty	7
Grape	<u>Vitis vinifera</u> L.	Reisling	20
Petunia	<u>Petunia hybrida</u> Vilm.	White cascade	3
Sugar beet	<u>Beta vulgaris</u> L.	U.S. H-10	10
Cornflower ²	<u>Centaurea cyanthus</u> L.	Blue boy	6
Tomato ²	<u>Lycopersicon esculentum</u> Mill.	Ace	8
TOLERANT [50-100% injury at 75 mg m ⁻³]			
Avocado	<u>Persea americana</u> Mill.	Hass; Bacon	60
Coreopsis	<u>Coreopsis grandiflora</u> Nutt.	Sunburst	3
Salvia	<u>Salvia splendens</u> Ker-Gawl	Patens	3
Wallflower	<u>Cheiranthus allioni</u> L.	Golden bedder	4
Barley ²	<u>Hordeum vulgare</u> L.	CM 67	4
VERY TOLERANT [50-100% injury at 150 mg m ⁻³]			
Briza	<u>Briza maxima</u> L.	Ornamental quaking grass	4
Cabbage	<u>Brassica oleracea</u> L.	<u>Capitata</u> c.v. Danish roundhouse	28
Citrus	<u>Citrus limo</u> (L.) Burm. J.	Roughlemon seedling	12
Citrus	<u>C. limo</u>	Lisbon lemon graft	80
Citrus	<u>C. sinensis</u> (L.) Osbeck	Valencia orange graft	80
Dudleya	<u>Dudleya caespitosa</u> (Haw.) Britt. & Rose		90
Pine	<u>Pinus muricata</u> D. Don.	Bishop pine	80
Poppy	<u>Eschscholtzia californica</u> Cham.	California poppy	27

¹Categories based on visible leaf area injury after 20-minute exposure to HCl gas at given concentration

²Limited data on these species

TABLE 9

LEAF AREA INJURED (%) ON PLANTS EXPOSED TO 20 MINUTES OF HCl AT
START, MIDDLE, OR END OF 90-MINUTE OZONE (O₃) EPISODES

Host Species	HCl Concn. (mg m ⁻³)	Ozone Concentration							
		0 pphm ¹	35 pphm ¹			0 pphm ²	50 pphm ²		
			Start	Middle	Timing of HCl dose End		Start	Middle	End
Pinto bean	0	0	68	64	48	0	61	80	88
	5	0	46	75	49	0	69	81	88
	10	24	29	63	57	33	79	74	88
	15	70	71	84	40	-	-	-	-
	20	84	85	58	48	88	88	85	88
	40	-	-	-	-	97	100	99	88
\bar{x} of treatments with O ₃			60±22	69±11	48±6		79±15	84±9	88±0
Grand Means		36±38		59±10		44±47		84±10	
Zinnia	0	0	2	7	4	0	27	35	32
	5	0	5	7	11	0	38	39	33
	10	2	4	8	5	0	20	41	39
	15	27	31	22	8	-	-	-	-
	20	40	52	25	15	49	34	43	24
	40	-	-	-	-	83	63	27	52
\bar{x} of treatments with O ₃			19±22	14±9	9±5		36±16	37±6	36±10
Grand Means		14±19		14±14		26±38		36±11	

¹Means of 4 replicates with 10 plants per treatment

²Means of 2 replicates with 10 plants per treatment

- Signifies no plants exposed to this treatment

Dry HCl gas quickly absorbs water to form aqueous hydrochloric acid. The ground cloud following a rocket launch contains great amounts of pollutants, particularly HCl (Goldford, 1976; Dawburn and Kinslow, 1976). If the cloud travels through fog, mist, or rain before dissipating, a unique HCl-rain could form. Several experiments were conducted to determine the effects an HCl-based acid precipitation would have on vegetation.

ACID MIST

Pinto beans were exposed to dry HCl gas for 20 minutes then were removed from chambers. HCl generation continued and the gas combined with a mist of distilled water created with an ultrasonic nozzle. A new set of bean plants

TABLE 10

DRY WEIGHTS (mg) OF PLANTS EXPOSED TO 20 MINUTES OF HCl AT
START, MIDDLE, OR END OF 90-MINUTE OZONE (O₃) EPISODES

Host Species	HCl Concn. (mg m ⁻³)	Ozone Concentration							
		0 ppmh ¹	35 ppmh ¹		0 ppmh ²		50 ppmh ²		
			Timing of HCl dose						
			Start	Middle	End		Start	Middle	End
Pinto bean	0	1335	843	1210	1163	1314	1122	756	720
	5	1427	926	1222	1176	1378	972	830	690
	10	1401	1185	1147	1137	1314	1206	866	768
	15	930	874	941	1205	-	-	-	-
	20	705	912	1190	999	592	844	708	602
	40	-	-	-	-	380	550	710	638
\bar{x} of treat- ments with O ₃			948+136	1142+116	1136+80		939+258	714+181	684+66
Grand Means		1160+324		1075+140		996+472		779+208	
Zinnia	0	442	418	397	420	454	358	298	274
	5	416	316	356	310	434	336	136	334
	10	306	283	374	359	408	306	244	252
	15	369	304	380	245	-	-	-	-
	20	310	316	339	273	190	334	292	274
	40	-	-	-	-	72	134	226	152
\bar{x} of treat- ments with O ₃			327+52	369+22	321+70		294+91	239+65	257+66
Grand Means		369+61		339+53		312+171		263+73	

1,2,- Same as for Table 9

was exposed to the HCl-mist mixture. Nearly 50% less leaf injury occurred when mist was present in the chamber compared to dry conditions even though the same amount of HCl entered the intake manifold (Table 15). With plants in the chamber, detectable HCl was reduced 88% under mist conditions, but was reduced 66% when plants were absent.

In the second experiment, HCl gas was metered into chambers being supplied with mist. Gas flows selected were those that produced 25 to 80% foliar injury on beans under dry conditions. Six pinto bean plants were exposed for 20

TABLE 11

SUMMARY OF ANALYSES OF VARIANCE COMPARING RESPONSES
OF BEANS AND ZINNIA PLANTS TO FUMIGATIONS OF HCl OR OZONE (O₃)

Pollutant	Response	Species	Means	df	F
HCl	Injury (%)	Bean	40+41	18	1.51
		Zinnia	20+29		
	Dry weight (mg)	Bean	1078+391	18	32.25*
		Zinnia	340+125		
O ₃	Injury (%)	Bean	51+34	14	8.32*
		Zinnia	13+15		
	Dry weight (mg)	Bean	1058+248	14	55.12*
		Zinnia	383+67		
HCl + O ₃	Injury (%)	Bean	50+19	46	74.37*
		Zinnia	27+17		
	Dry weight (mg)	Bean	917+236	46	157.05*
		Zinnia	286+70		

*Species significantly different at 5% level by analysis of variance

TABLE 12

SUMMARY OF RESPONSES OF PLANTS EXPOSED TO GASEOUS
HCl, OZONE (O₃), or HCl PLUS OZONE

Response	HCl Concentration (mg m ⁻³)	Bean O ₃ Conc. (pphm)			Zinnia O ₃ Conc. (pphm)		
		0	35	50	0	35	50
Injury (%)	0	0	60	76	0	4	31
	5	0	57	79	0	8	37
	10	28	50	80	1	6	33
	15	70	65	-	27	20	-
	20	86	64	87	44	30	34
	40	97	-	96	83	-	47
Dry weight (mg)	0	1024	1072	866	388	412	310
	5	1402	1108	831	375	327	269
	10	1358	1156	947	346	339	267
	15	930	1007	-	304	310	-
	20	648	1034	718	253	309	300
	40	380	-	533	72	-	171

- signifies no plants exposed to this treatment

TABLE 13

LINEAR REGRESSION COEFFICIENTS RELATING GAS CONCENTRATIONS
TO RESPONSE OF BEAN AND ZINNIA PLANTS TO HCl OR OZONE (O₃) FUMIGATIONS

Pollutant	Response	Species	Correlation Coefficient (r)	t
HCl	Injury	Bean	0.906	6.05*
		Zinnia	0.955	9.01*
	Dry weight	Bean	-0.904	5.97*
		Zinnia	-0.938	7.65*
O ₃	Injury	Bean	0.956	7.97*
		Zinnia	0.788	3.11*
	Dry weight	Bean	-0.752	2.80*
		Zinnia	-0.810	3.38*
HCl ¹ + O ₃	Injury	Bean	0.408	2.10*
			(0.716)	(4.81*)
		Zinnia	0.514	2.81*
			(0.653)	(4.04*)
	Dry weight	Bean	-0.623	3.74*
			(-0.764)	(5.54*)
		Zinnia	-0.530	2.93*
			(-0.509)	(2.78*)

¹First row of statistics is of 4 HCl concentrations with all O₃ treatments; second row (in parenthesis) is of 2 O₃ concentrations with all HCl treatments

*signifies t-test is within 95% confidence interval

minutes at each flow setting and were graded 24 hours after exposure (Table 16). Leaf area injury was less than expected. Bubbler samples taken during exposure indicated that a small gaseous HCl concentration existed (avg. 4.2 mg m⁻³); this helped to explain the relative lack of injury (avg. 10.4%). HCl concentration correlated poorly with flow rate ($r = 0.49$, $F = 2.56$, N.S.), with injury ($r = -0.19$, $F = 0.30$, N.S.), or with temperature ($r = 0.00$, $F = 0.00$, N.S.). A significant negative linear correlation was found between flowrate and injury ($r = 0.73$, $F = 9.37$, $p = 5\%$).

TABLE 14

ANALYSES OF LEAF AREA INJURY AND HARVESTED DRY WEIGHTS OF
BEAN AND ZINNIA PLANTS EXPOSED TO HCl FOR 20 MINUTES
DURING 90-MINUTE OZONE (O₃) EPISODES

Response	Species	O ₃ Concn. (pphm)	Grand Means	F-value	
				HCl Concn. df = 4,8 F5% = 3.85	Time of HCl ¹ df = 2,8 F5% = 4.46
Injury (%)	Bean	35	59+10	1.99	0.43
		50	84+10	1.28	2.47
	Zinnia	35	14+14	1.33	4.00*
		50	36+11	0.01	0.82
Dry weight (mg)	Bean	35	1075+140	4.35	0.76
		50	778+208	7.34*	5.82*
	Zinnia	35	339+53	2.47	3.92
		50	263+73	0.98	2.31*

¹HCl applied to plants for 20 minutes at start, middle, or end of
O₃ episode

*F significant at 5% level

TABLE 15

DETECTABLE HCl AND LEAF INJURY UNDER MIST AND DRY CONDITIONS

Chamber Condition	HCl Flow Setting	HCl Concentration (mg m ⁻³)	1 Pinto Bean Injury	
			No. Leaves (%)	Leaf Area (%)
dry	53	20	100	99
dry	53	24	100	98
mist ²	53	2	100	40
mist	53	3	100	55
dry	53	18	--	--
mist	53	6	--	--

¹Beans exposed 20 minutes and graded after 24 hours

²Mist condition created with distilled water forced through an ultra-
sonic nozzle

- signifies no plants were present

TABLE 16
AVERAGE INJURY ON PINTO BEAN PLANTS EXPOSED TO GASEOUS
HCl UNDER MIST CONDITIONS

Gas Flow Setting	HCl Concentration (mg m ⁻³)	Leaf Area Injured (%)	Temperature (°C)
49.5	3.3	12.5 ± 0.0	27
50.0	4.6	11.5 ± 1.6	27
50.5	3.2	10.4 ± 2.6	25
51.0	2.2	10.4 ± 2.6	24
51.5	4.3	10.4 ± 2.6	23
52.0	5.9	10.4 ± 1.6	27
52.5	4.0	8.9 ± 2.4	27
53.0	3.6	10.9 ± 2.6	25
53.5	4.3	8.3 ± 1.6	24
54.0	6.1	9.9 ± 2.4	23

¹Mean injury on 6 plants exposed per concentration; all exposed leaves injured

Water mist decreased chamber HCl gas concentration by scrubbing gas from the atmosphere. The scrubbed gas became an acid mist which coated all leaf surfaces. Resulting injury was less severe than that caused by dry gas without mist. In further work, acid solutions were prepared and sprayed directly on plants to simulate natural rain.

ACID SPRAY

General Effects

In a preliminary trial, solutions of 0.5 to 25% HCl (v/v) in distilled-deionized water were sprayed with a plastic misting bottle onto pinto bean plants until the leaves dripped. All primary leaves exhibited injury on the following day (Table 17). Necrotic spots formed on leaves treated with weak solutions (0.5-3%) while a coalescing brown necrosis resulted with stronger concentrations (5-25%). Necrotic area and degree of coalescence depended on acid concentration.

Concentration Effects

Acid solutions were sprayed onto 14-day-old bean and 21-day-old zinnia plants. Weaker solutions did not visibly injure all leaves (Table 18). Zinnia leaves, which had necrotic spotting similar to that seen on beans, were less sensitive than bean primary leaves. No single zinnia leafset received more injury than any other (Table 19).

TABLE 17
INJURY ON 16-DAY-OLD PINTO BEAN PRIMARY LEAVES SPRAYED WITH
HCl

HCl Concentration (%)	Leaf Area Injured (%)
0.5	25
1	50
3	90
5	100
10	100
15	100
20	100
25	100

TABLE 18
FOLIAR INJURY ON BEAN AND ZINNIA PLANTS SPRAYED WITH HCl

HCl Concentration (%)	Pinto Beans ¹		Zinnia ²	
	No. Leaves (%)	Leaf Area (%)	No. Leaves (%)	Leaf Area (%)
0.1	83	5.2 ± 1.8	79	7.8 ± 1.3
0.2	100	9.4 ± 3.1	96	10.4 ± 0.5
0.3	100	9.4 ± 3.1	96	12.2 ± 0.5
0.5	100	19.4 ± 1.8	100	24.1 ± 4.2
1.0	100	37.5 ± 25.0	100	28.9 ± 6.7
2.0	100	87.5 ± 0.0	100	70.0 ± 10.2
3.0	100	87.5 ± 0.0	100	83.3 ± 4.8
5.0	100	87.5 ± 0.0	100	87.5 ± 0.0

¹Three plants, each with 2 primary leaves, sprayed at each concentration

²Three plants, each with 4 leafsets, sprayed at each concentration

Year-old citrus seedlings (*Citrus sinensis* c.v. Sweet Orange) exhibited white necrotic leaf spots three days after being sprayed with aqueous HCl (Table 20). Although the 1-7% HCl treatments caused lesion coalescence and abscission of many leaves, others were uninjured.

TABLE 19
LEAFSET INJURY ON ZINNIA PLANTS SPRAYED WITH HCl

Leafset Number ¹	Leaf Area Injured (%)
1	36 ± 38
2	39 ± 34
3	43 ± 33
4	40 ± 34
¹ Oldest leafset is #1; average of 48 leaves	

TABLE 20
EFFECT OF HCl SPRAYS ON CITRUS SEEDLING LEAVES

HCl Concentration (%)	Injury		
	No. Leaves (%)	Leaf Area (%)	Leaf Drop (%)
0.05	23.6	1.1 ± 0.5	0
0.10	39.3	2.5 ± 2.5	0
0.50	68.2	8.0 ± 5.2	0
1.00	88.7	14.0 ± 0.6	0
5.00	89.1	21.0 ± 1.7	25
7.00	75.3	13.0 ± 9.1	16

Short Term Biomass Effects

Bean and zinnia plants sprayed with 10-fold dilutions of HCl or with a water control were graded for injury 24 hours later (Table 21). Plant tops harvested one week after spraying were dried and weighed. Reductions in dry weights of acid-treated plants compared to water-controls were calculated (Table 21).

The 0.01% HCl treatment did not injure leaves of either species nor affect plant weights. Both zinnia and beans were injured by 0.1% HCl treatment and, although bean plant weight decreased 14% compared to the control, zinnia weight was unchanged. When sprayed with 1.0% HCl, biomass reduction for zinnias was greater than for beans.

TABLE 21
INJURY AND BIOMASS REDUCTION IN PLANTS SPRAYED WITH HCl

HCl Concentration (%)	Pinto Bean			Zinnia		
	Injury		Dry Wt. Reduc. ¹ (%)	Injury		Dry Wt. Reduc. ¹ (%)
	No. Leaves (%)	Leaf Area (%)		No. Leaves (%)	Leaf Area (%)	
0.01	3	0.0	0	0	0.0	0
0.10	100	7.9	14	100	10.1	0
1.00	100	23.7	24	100	72.1	51

¹Biomass reduction = [(control - treated)/control] x 100%

Comparison of HCl and H₂SO₄ as Sprays

Since acid rain more often contains dilute H₂SO₄ than HCl, comparing the phytotoxicity of the two acids seemed appropriate. Pinto bean and zinnia plants were sprayed with a dilution series of HCl or H₂SO₄ and were graded for visible injury on the following day. Plant tops harvested one week after spraying were dried and weight reductions over water-controls were calculated.

H₂SO₄ caused more visible injury than HCl (Table 22). Zinnias were more sensitive than bean plants to biomass reduction from acid spray treatments. With zinnia plants, both acids equally reduced biomass at the 0.5 or 1% spray levels, but bean biomass was reduced more with H₂SO₄ than with HCl.

TABLE 22
LEAF INJURY AND TOP DRY WEIGHT REDUCTION OF PLANTS SPRAYED WITH HCl OR H₂SO₄

Acid Concn. (%)	Pinto Bean				Zinnia			
	HCl		H ₂ SO ₄		HCl		H ₂ SO ₄	
	Injury (%)	Dry Wt. ¹ Reduction (%)	Injury (%)	Dry Wt. Reduction (%)	Injury (%)	Dry Wt. Reduction (%)	Injury (%)	Dry Wt. Reduction (%)
0.01	0	0	6	7	2	20	3	2
0.05	3	0	16	11	9	26	13	14
0.10	6	19	62	33	12	15	55	38
0.50	22	20	88	57	73	65	83	64
1.00	78	31	88	56	77	71	85	71

¹Dry weights compared to weights of water-sprayed controls

Effect of Plant Age on Sensitivity to Acid Spray

Age often influences plant sensitivity to gaseous air pollutants (Hanson, et al., 1975). Endress, Oshima, and Taylor (1979) found pinto beans most sensitive to injury from exposure to short periods of HCl gas when 12 to 14 days old and susceptibility decreased in younger and older plants. Zinnias were most sensitive to HCl when 3 weeks old (Granett and Taylor, 1978). Bean and zinnia plants of different ages were sprayed with HCl simultaneously to determine whether susceptibility to this toxicant varied with plant age. Spray concentrations and plant ages were changed for a second trial.

For either species, injury was significantly different at the different concentrations (Tables 23 and 24). Sensitivity of zinnia plants was not affected by age nor was interaction between age and concentration significant (Table 24). Concentration, age, and age vs. concentration interaction effects, however, were significant for beans. Separation of injury means based on concentration differences was clear in the first trial, but no meaningful age-concentration relationships could be defined (Table 23). Analysis of the second trial, where range of ages was greater, indicated that the oldest plants were significantly less injured than plants of other ages. Age was not useful in predicting plant sensitivity to HCl sprays.

TABLE 23
EFFECT OF PLANT AGE ON SENSITIVITY TO INJURY FROM HCl SPRAYS ON BEAN
PRIMARY LEAVES

Age (days)	Trial 1			Trial 2		
	HCl Concentration			HCl Concentration		
	0.1%	1.0%	\bar{x}	0.02%	0.75%	\bar{x}
8	12 ¹	73	42 ² x	-	-	
10	12	52	32 y	6	88	47 y
12	11	35	23 yz	5	82	44 y
13	11	21	16 yz	-	-	
14	12	52	32 y	4	82	43 y
15	15	88	51 x	-	-	
16	12	43	27 y	5	70	37 y
17	14	85	49 x	-	-	
18	-	-		4	68	36 y
20	-	-		1	75	38 y
24	-	-		1	39	20 z

¹Avg. leaf area injured for 5 plants

²Data followed by the same letter not significantly different at 1% level by Duncan's Multiple Range Test

- signifies no plants treated

TABLE 24

EFFECT OF PLANT AGE ON SENSITIVITY TO INJURY FROM HCl SPRAYS ON ZINNIA LEAVES

Age (days)	Trial 1		Trial 2	
	HCl Concentration		HCl Concentration	
	0.1%	1.0%	0.02%	0.75%
16	-	-	0	75
18	-	-	0	61
19	14 ¹	83	-	-
20	-	-	2	62
21	19	86	-	-
23	15	85	-	-
24	-	-	3	70
25	16	87	-	-
26	-	-	2	57
28	-	-	5	63
30	-	-	4	63

¹Avg. leaf area injured (%) for 5 plants

- signifies no plants treated

Diurnal Phytotoxicity of Acid Spray

Granett and Taylor (1980b) observed that plants sustained greater injury during 20-minute exposures to gaseous HCl at a given concentration at midday than in the early morning or late afternoon. A study was conducted to determine whether diurnal influences affected plant responses to aqueous HCl sprays.

Groups of four pinto bean and four zinnia plants were sprayed with 0.1, 0.5, or 1.0% HCl solutions every 2 hours from 0600 (6 a.m.) to 1800 (6 p.m.) Pacific Standard Time. Temperature (T), light (PAR = photosynthetically active radiation; LI = light intensity), and relative humidity (RH) were recorded hourly. Injury symptoms developed on all treated leaves within 24 hours, at which time amounts of injured leaf areas were estimated (Table 25). A week after spraying, plant tops were harvested, dried, and weighed (Table 26).

Acid concentration strongly influenced leaf injury and biomass. Environmental data for T, RH, PAR, and LI approximated normal curve distributions over the 12-hour period (Table 27 and Figure 13). Significant positive linear correlations existed between PAR and LI ($p < 5\%$), PAR and T ($p < 5\%$), and PAR and RH ($p < 5\%$) (Table 28). No significant correlations related injury and biomass responses for both species with any of the environmental values, nor were injury and biomass responses related (Table 28). Species response was compared by correlating injury and biomass at each spray concentration (Table 29). Injury of pinto beans was not significantly correlated with

TABLE 25

LEAF INJURY ON PLANTS SPRAYED WITH HCl OVER A DIURNAL PERIOD

Time of Day (hour)	Pinto Bean			Zinnia			Average Injury (all concns.)
	<u>HCl Concentration</u>			<u>HCl Concentration</u>			
	0.1%	0.5%	1.0%	0.1%	0.5%	1.0%	
0600	11 ¹	28	45	14	62	84	41+29
0800	10	34	78	14	48	83	45+31
1000	11	30	84	15	37	83	43+32
1200	12	39	81	12	41	83	45+32
1400	12	16	75	12	66	86	45+35
1600	10	52	88	12	62	81	51+33
1800	11	22	72	12	31	81	38+31
Avg. Injury (all times)	11+1	32+12	74+14	13+1	50+14	83+2	

¹Avg. leaf area injured (%) of 4 plants

TABLE 26

BIOMASS OF PLANTS SEVEN DAYS AFTER ONE HCl SPRAY

Time of Day (hour)	Pinto Bean			Zinnia			Average Biomass (all concns.)
	HCl Concentration			HCl Concentration			
	0.1%	0.5%	1.0%	0.1%	0.5%	1.0%	
0600	1790 ¹	1395	895	610	292	162	857+636
0800	1775	1445	1275	570	532	187	964+622
1000	1672	1297	557	695	342	182	791+578
1200	1780	1215	935	632	387	242	865+572
1400	1692	1422	1100	552	207	240	869+628
1600	1435	1315	552	610	220	170	717+540
1800	1645	1442	972	637	427	142	878+586
Average Biomass (all times	1684+124	1362+88	898+266	615+47	344+116	189+38	

¹Avg. top dry weight (mg) of 4 plants

TABLE 27

ENVIRONMENTAL VARIABLES DURING 12-HOUR PERIOD AND RESPONSE OF
PLANTS SPRAYED WITH HCl DURING THAT PERIOD

Time (hours)	Light		Temperature (°C)	RH ³ (%)	Injury ⁴ (%)	Biomass ⁵ (mg)
	PAR ¹	LI ²				
0600	0	0	22	35	41	857
0700	3	10	20	37		
0800	62	10	22	38	44	964
0900	400	18	22	38		
1000	660	21	25	40	44	791
1100	760	24	28	41		
1200	870	45	29	41	45	865
1300	940	42	29	39		
1400	680	28	31	41	44	869
1500	415	15	32	40		
1600	87	5	25	40	51	717
1700	1	2	23	35		
1800	0	0	23	40	38	878

¹PAR, photosynthetically active radiation in μ -einsteins $m^{-2} sec^{-1}$

²LI, light intensity in 10^5 ergs $cm^{-2} sec^{-1}$

³RH, relative humidity

⁴Avg. of leaf area injured on 4 bean and 4 zinnia plants

⁵Avg. dry weight of tops of 4 bean and 4 zinnia plants harvested 7 days after spray treatment

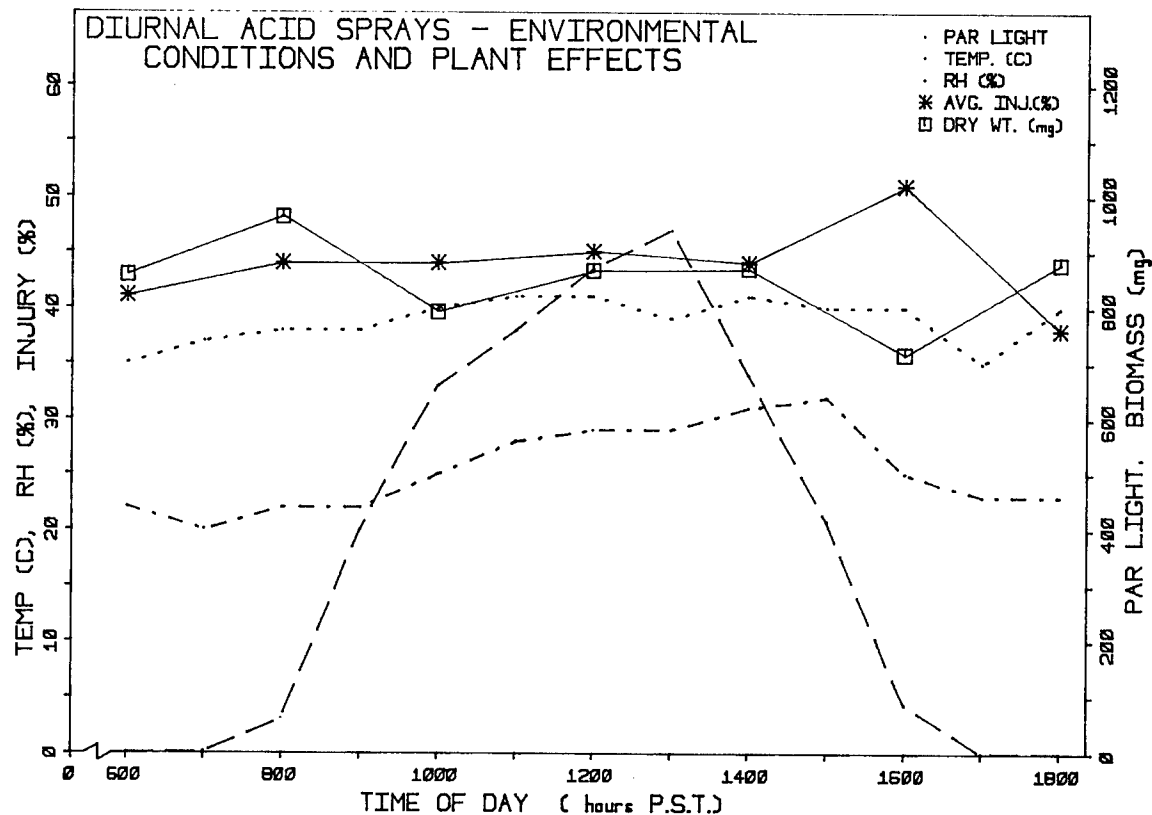


Figure 13. Relations between average responses of plants to acid spray and environmental variables of light, temperature, and humidity.

TABLE 28
STATISTICAL ANALYSIS OF ENVIRONMENT DATA AND PLANT RESPONSE AFTER
ACID SPRAY DIURNAL EXPERIMENT

Factors ¹	LI	T	RH	Injury	Biomass
PAR	r= 0.94 ² F=84.03 ***	r= 0.74 F=13.62 ***	r= 0.65 F= 7.96 **	r= 0.20 F= 0.21 NS	r= 0.08 F= 0.03 NS
LI	-	r= 0.65 F= 8.11 **	r= 0.56 F= 5.15 *	r= 0.24 F= 0.30 NS	r= 0.08 F= 0.03 NS
T	-	-	r= 0.68 F= 9.71 ***	r= 0.30 F= 0.51 NS	r=-0.24 F= 0.12 NS
RH	-	-	-	r= 0.30 F= 0.49 NS	r=-0.24 F= 0.31 NS
Injury	-	-	-	-	r=-0.60 F= 2.87 NS

¹See Table 27 for abbreviations; injury and biomass analyses include all plants of both species

²r, regression coefficient; F, F-value; ***, p<1%; **, p<5%; *, p<10%; NS = not significant, p>10%; - signifies impossible or duplicate comparison

TABLE 29
ANALYSIS OF PLANT DIURNAL RESPONSES COMPARING BIOMASS AND LEAF AREA
INJURED AT EACH SPRAY CONCENTRATION

Statistic ¹	Pinto Bean HCl Concentration					Zinnia HCl Concentration			
	0.1%	0.5%	1.0%	\bar{x}	\bar{x}	0.1%	0.5%	1.0%	\bar{x}
r	-0.59	0.43	-0.57	-0.28	-0.60	0.38	-0.69	0.65	-0.79
F	2.69	1.14	2.47	0.43	2.85	0.86	4.57	3.65	8.48
	NS	NS	NS	NS	NS	NS	*	NS	**

¹Seven pairs of data (biomass vs. injury) were compared for linear regression (r = correlation coefficient) and significance (F-value)

NS, *, ** See Table 28

spray concentrations. Biomass decreased as injury increased when zinnia plants were treated with 0.5% HCl spray ($p < 10\%$) or when the average of the three spray treatments was analyzed ($p < 5\%$). The two significant zinnia responses were not significantly correlated with any of the environmental variables tested (Table 30).

Plant response to acid sprays was not affected by diurnal environmental variables.

Cumulative Effects of Acid Sprays

Response to repeated doses of a gaseous pollutant depends on the plant species and the pollutant. Partial to complete recovery between episodes minimizes injury allowing plant growth and yield to be relatively unaffected. With low-concentration, long-term doses of HF, however, the toxicant can accumulate until visible injury results (Jacobson et al., 1966).

One, two, or three daily applications of 0.01, 0.1, or 1.0% solutions of HCl or H_2SO_4 were sprayed on bean and zinnia plants. Injury was recorded 24 hours after final spray and 7 days after treatment, plant tops were harvested, dried, and weighed.

TABLE 30
ANALYSIS OF ZINNIA RESPONSE FROM HCl SPRAY TREATMENT COMPARED TO
THREE ENVIRONMENTAL FACTORS¹

Environmental Factor	0.5% Spray		Average Response ²	
	Injury	Biomass	Injury	Biomass
PAR	$r = 0.10^3$ $F = 0.05$ NS	$r = -0.21$ $F = 0.23$ NS	$r = -0.04$ $F = 01$ NS	$r = 0.13$ $F = 0.09$ NS
T	$r = 0.24$ $F = 0.30$ NS	$r = 0.50$ $F = 1.68$ NS	$r = 0.26$ $F = 0.37$ NS	$r = 0.28$ $F = 0.44$ NS
RH	$r = -0.29$ $F = 0.45$ NS	$r = -0.14$ $F = 0.10$ NS	$r = -0.29$ $F = 0.45$ NS	$r = 0.01$ $F = 0.02$ NS

¹See Tables 27 and 28 for abbreviations

²Avg. of responses for 0.1, 0.5, and 1.0% HCl sprays

³Seven paired means compared in each analysis

Amount of visible response increased with acid concentration (Table 31), but injury could not be predicted by number of spray applications. In bean plants, three sprays were usually more injurious than one. Zinnia injury increased with increasing number of applications, except at the lowest HCl levels where injury was slight and highest H₂SO₄ levels where plant death occurred. In most (83%) cases, H₂SO₄ was more injurious than HCl for the same species-concentration-number of applications treatment. Beans were more sensitive than zinnias to 89% of the H₂SO₄ spray treatments and to 56% of the HCl treatments.

Since plant tops were harvested 7 days after the last spray application (Table 32), plants treated with one spray were 3 days younger at harvest than plants sprayed three times so dry weight comparisons may not be valid. Although plants sprayed with 1.0% acid yielded less biomass than those receiving 0.01% acid, further generalizations concerning the dry weights of plants sprayed with intermediate acid levels could not be made. H₂SO₄ at the highest spray concentrations caused more biomass reduction than HCl (except for zinnias sprayed three times).

This experiment showed that strong (1%) acid sprays severely injured plants and caused reductions in plant biomass detectable one week after treatments. Acid sprays generally produced progressively more injury as the number of applications increased as long as acid concentrations remained below levels that killed plants. Final yield depended on acid concentration, cumulative damage, and time interval between last spray treatment and harvest.

In another trial, marigolds (*Tagetes patula* c.v. Goldie) were sprayed weekly with HCl or H₂SO₄. On the fifth week plant tops were harvested, dried, and weighed (Table 33). Small acid concentrations (below 0.38%) did not injury plants. Largest acid concentrations severely damaged or killed young plants and the first application was not repeated. H₂SO₄ reduced plant biomass at smaller spray concentrations than HCl.

TABLE 31
LEAF AREA INJURED (%) ON PLANTS SPRAYED ONE TO
THREE TIMES WITH ACID SOLUTIONS

Number of Treatments	HCl			H ₂ SO ₄		
	0.01%	0.1%	1.0%	0.01%	0.1%	1.0%
<u>Bean Plants</u>						
1	2	10	72	6	41	88
2	6	14	81	9	41	100
3	6	12	72	10	59	88
<u>Zinnia Plants</u>						
1	1	9	82	2	8	44
2	4	11	88	5	25	100
3	3	15	91	7	41	100

TABLE 32

BIOMASS (dry weight in mg) OF PLANT TOPS HARVESTED
SEVEN DAYS AFTER ONE TO THREE ACID SPRAY TREATMENTS

Number of Treatments	HCl			H ₂ SO ₄		
	0.01%	0.1%	1.0%	0.01%	0.1%	1.0%
<u>Bean Plants</u>						
1	635	640	442	580	555	370
2	855	790	442	762	588	312
3	792	822	468	845	785	402
<u>Zinnia Plants</u>						
1	148	88	75	140	118	38
2	225	155	58	110	135	38
3	218	210	62	210	92	82

TABLE 33

DRY WEIGHT (g) OF MARIGOLDS EXPOSED TO FOUR WEEKLY ACID SPRAYS AND
HARVESTED ON FIFTH WEEK

Acid concentration (%)	HCl	H ₂ SO ₄
0	1.60	1.19
0.003	--	1.53
0.03	1.67	1.42
0.38	1.11	0.05
0.75	0.21	0.02
1.5 ¹	0.05	0.03
3.0 ¹	0.02	dead

¹Plants sprayed on first week only

- signifies plants not treated at this dose

PHYTOTOXICITY OF GASEOUS HYDROGEN FLUORIDE

CHECKS OF FLUORIDE SYSTEMS

Generator and Sampler

The generator functioned satisfactorily when 24.6% HF solution flowed at a rate of 0.24 ml per minute to the 100 °C volatilization oven. Nitrogen carrier gas flowed through the systems at 12.5 liters per minute. To test the sampling system, 20 liters were drawn from the exposure chamber through two plastic bubblers in series; each bubbler contained 20 ml distilled water adjusted to pH 10.9. Measurable amounts of HF never accumulated in the second bubbler. Chamber concentration stabilized after 30-35 minutes operation and rapidly declined when HF generation ceased (Table 34). During subsequent tests, gas production preceded chamber use by 30 to 90 minutes.

Safety Tests

When air samples were taken with a bubbler from the exposure chamber, from the adjacent no-gas control chamber, and from the exhaust-fan output, no significant HF in the control chamber or at the exhaust was detected (Table 35).

ANALYSIS TECHNIQUES

Analysis for Fluoride

Analysis for fluoride in aqueous solutions with the specific-ion electrode was satisfactory, with little variability in replicate determinations of the same sample. The procedure for analysis of fluoride in

TABLE 34

DETECTION OF HF GAS IN AN EXPOSURE CHAMBER USING TWO SAMPLERS IN SERIES

Time after Start of Generator (minutes)	HF Concentration (mg m ⁻³)	
	First Bubbler	Second Bubbler
9	18.3	0.04
20	22.5	0.04
32	25.0	0.04
43	30.2	0.04
53 ¹	28.5	0.04
64	17.9	0.04
76	5.2	0.04

¹HF generation ceased after 55 minutes

TABLE 35
MEASUREMENT OF HF IN AND AROUND EXPOSURE CHAMBER

Location	Time after Start of Generator (minutes)	HF Concentration (mg m ⁻³)
Exposure chamber	30	18.0
Exhaust	35	0.2
Exposure chamber	75	14.5
Control chamber	80	0.2
Exhaust	70	0.2

tissue was standardized. To test the variability in the technique, dried leaf tissue from a severely injured 20-day-old pinto bean plant exposed to 3.5 mg HF m⁻³ for 20 minutes was weighed into 0.05 g aliquots. After ashing in an oxygen atmosphere, the adjusted absorbing solution was measured for fluoride. Six samples contained an average of 664 ± 52 $\mu\text{g F}^-$ per g tissue.

Fluoride Activity After Storing Liquid Samples

To determine whether fluoride concentrations of aqueous samples change during storage, three 20-liter air samples were drawn from the same HF chamber. Each 20 ml solution was stored in a sealed plastic vial at laboratory temperatures (25-27°C) and checked three times for activity. Total ionic strength adjusting buffer (TISAB) was added to one vial immediately after collection and to aliquots of the other two vials just prior to analysis. No change in HF concentration was noted after storing the samples up to nine days nor did buffer technique cause significant changes in detectable HF (Table 36).

TABLE 36
HF (mg m⁻³) IN AIR SAMPLES STORED WITH OR WITHOUT BUFFER FOR 0 TO 9 DAYS

Vial	Storage	DAY ANALYZED		
		0	4	9
1	No TISAB	2.75	2.72	2.73
2	No TISAB	2.28	2.28	--
3	With TISAB	2.30	2.28	2.33

- signifies no sample for this treatment

Plant Variability

Groups of ten 33-day-old zinnia and ten 19-day-old bean plants were exposed to two concentrations of HF for 25 minutes. All bean primary leaves and 85-86% of all zinnia leaves showed visual injury the day after exposures (Table 37). Pinto beans exhibited significantly more leaf area injury ($p = 5\%$) after exposure to 5.2 mg HF m^{-3} than to 3.9 mg m^{-3} , but no statistical differences were noted in zinnia response. Likewise, no significant difference existed between the average amount of injury received by beans compared to zinnias.

TABLE 37
LEAF INJURY ON PLANTS EXPOSED TO HF GAS FOR 25 MINUTES

HF Concentration (mg m^{-3})	Pinto Bean			Zinnia	
	No. Leaves Injured (%)	Leaf Area Injured (%)		No. Leaves Injured (%)	Leaf Area Injured (%)
3.9	100	25.0 ¹	b	85	48.4 a
5.2	100	57.8	a	86	55.3 a

¹Means followed by the same letter are not significantly different at 1% level by an analysis of variance

Chamber Variability

HF concentration fluctuated even when the chamber remained empty and closed. To test this variability empty chambers were operated at two different HF concentrations over a 5-day period. Two pumps drove single 20-ml plastic syringes containing 17% HF solution at delivery rates of 0.61 (low) and 0.91 ml per minute (high). The syringe pumps were activated an hour after the oven was started. After allowing 60 minutes for the generator to stabilize, three air samples were drawn from each chamber (Table 38). Bartlett's test for homogeneity of variances performed on valid concentration replicates determined that the daily between-replicate (within-run) variances differed at the 5% level of significance. The problem of heterogeneity in variances between same-day replicates had to be resolved before concentration means could be compared and analyzed.

HF monitoring continued for two more days after adjusting the gas delivery lines (Table 39). Bartlett's test indicated within-run heterogeneity was not significant at the 5% level. Further statistical analysis indicated that HF concentration varied between chambers at the 5% level but did not vary significantly among days, time of day, or analytical procedure.

The generation system, particularly the method of introducing acid for vaporization, was thought to be responsible for much variability in

TABLE 38
VARIABILITY IN HF GAS CONCENTRATIONS (mg m^{-3}) IN
CHAMBERS DURING FIVE-DAY TRIAL

HF Concn.	Replicate	DAY									
		1		2		3		4		5	
		AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
Low	1	9.6	6.6	0.6 ¹	7.5	4.2	4.6	7.0	0.3 ¹	7.8	5.8
	2	5.6	10.1	0.4	8.0	7.7	6.6	8.6	0.1	6.7	5.1
	3	<u>10.9</u>	<u>9.1</u>	<u>0.3</u>	<u>9.3</u>	<u>9.4</u>	<u>8.6</u>	<u>7.9</u>	<u>0.1</u>	<u>7.7</u>	<u>10.5</u>
	\bar{x}	8.7	8.6		8.2	7.1	6.6	7.8		7.4	7.1
	Sd	2.7	1.8		0.9	2.6	2.0	0.8		0.6	2.9
High	1	15.1	6.8	11.0	14.9	5.1	9.9	13.6	10.3	4.4	10.6
	2	13.2	12.5	9.3	8.1	5.4	10.5	13.7	9.4	13.4	9.9
	3	<u>11.3</u>	<u>10.2</u>	<u>9.5</u>	<u>11.4</u>	<u>5.0</u>	<u>13.5</u>	<u>12.0</u>	<u>10.3</u>	<u>12.8</u>	<u>9.1</u>
	\bar{x}	13.2	9.8	9.9	11.5	5.2	11.3	13.1	10.0	10.2	9.9
	Sd	1.9	2.9	0.9	3.4	0.2	1.9	1.0	0.5	5.0	0.7

¹Very low values indicate system malfunction, these data not analyzed

TABLE 39
VARIABILITY IN HF GAS CONCENTRATIONS (mg m^{-3})
IN CHAMBERS DURING TWO-DAY TRIAL

HF Concn.	Replicate	DAY			
		1		2	
		AM	PM	AM	PM
Low	1	7.3	7.1	6.5	6.1
	2	5.8	5.9	5.6	5.1
	3	<u>5.7</u>	<u>6.1</u>	<u>5.1</u>	<u>7.1</u>
	\bar{x}	6.3	6.4	5.7	6.1
	Sd	0.9	0.7	0.7	1.0
High	1	9.2	11.8	9.7	8.7
	2	10.5	13.1	9.2	10.2
	3	<u>12.1</u>	<u>10.6</u>	<u>8.9</u>	<u>14.3</u>
	\bar{x}	10.6	11.8	9.3	11.1
	Sd	1.5	1.2	0.4	2.9

chamber concentration. Although glass syringes worked more smoothly than plastic disposable syringes, HF acid reacted with the glass and a residue collected in the generator tubes. Plastic syringes worked adequately if chosen with care to assure smooth, consistent operation.

PLANT TOXICITY

Species Reaction to Short Exposures

Seven plant species were exposed to HF gas. Weather, which varied from hot and sunny days to overcast skies with cold and rain, affected both plant growth and sensitivity. Despite these additional stresses, useful information on species reaction to HF was collected (Table 40). Barley plants were resistant to HF injury while pinto beans were sensitive. Dudleya, with no visible injury after 20 minute exposures to 0.2, 4.0, 10.0, or 17.3 mg HF m⁻³, was the most resistant species tested.

Seed Reaction to Short Exposures

Seeds exposed to HCl gas responded to the treatment only when media on which they germinated had been exposed to and had adsorbed HCl (Granett and Taylor, 1980a). Seeds were expected to react similarly to HF exposures.

TABLE 40
LEAF AREA INJURED (%) ON PLANTS
EXPOSED TO HF GAS FOR 20 MINUTES

HF Concn. (mg m ⁻³)	Barley (28)	Zinnia (28)	Marigold (28)	Lettuce (28)	Radish (28)	Tomato (28)	Bean (14)
0.2	0	0	0	0	0	5.2	0
1.2	0	0	0	0	0	0	0
3.3	9.2	29.2	18.7	41.7	5.2	31.3	16.7
4.0	11.8	3.1	6.2	25.0	7.8	39.6	9.4
4.7	43.4	66.7	53.9	54.2	48.2	60.4	83.3
7.0	48.2	73.6	77.4	59.7	67.3	66.7	87.5
9.5	40.3	78.8	51.0	61.1	63.5	70.8	83.3
9.6	39.1	67.2	79.9	80.6	75.6	83.3	87.5
9.7	56.4	38.3	69.4	73.6	87.5	87.5	83.3
10.0	44.6	34.7	58.6	47.2	71.5	75.0	85.4
13.3	55.8	72.1	76.4	71.0	85.8	83.3	87.5
13.7	65.9	81.2	87.0	81.9	82.1	87.5	87.5
14.9	76.9	66.1	64.6	62.5	75.0	83.3	87.5
17.3	63.1	69.4	87.5	81.9	79.2	87.5	87.5
18.9	68.0	33.9	82.6	76.4	85.4	79.2	87.4

Tomato (*Lycopersicon esculentum* c.v. Ace) seeds and filter paper discs were arranged in four treatment groups: (A) seeds and filter paper both exposed to 9.5 mg HF m⁻³ for 20 minutes; (B) unexposed seeds placed on HF-exposed filter paper; (C) seeds exposed to HF placed on unexposed filter paper; and (D) unexposed controls of both seeds and filter paper. Each treatment consisted of 20 seeds and was replicated three times. Seeds and filter paper were treated in open Petri plates and, after transfers to form groups, plates were covered. Water was added to the plates before storing them in the dark at 19-22°C.

Percent seed germination (PSG) and seedling lengths were measured after 144 hours (Table 41). Exposing the paper support media to HF completely inhibited germination. Significantly reduced PSG and seedling lengths occurred after exposing only seeds and not support media. Thus seeds were more sensitive to HF gas than to HCl.

In a similar test, tomato seeds were exposed to 8 mg HF m⁻³ for 20 minutes with or without filter paper. A fifth treatment consisted of rinsing sets of exposed seeds in distilled water after exposure. Seeds were incubated on filter paper discs in Petri plates and, after 144 hours, PSG and total seedling lengths were recorded (Table 42). Unexposed control seeds developed best. Exposure of seeds or filter paper to HF reduced both PSG and lengths. Exposed rinsed seeds had higher PSG and greater seedling lengths than exposed unrinsed seeds. HF apparently adsorbed vigorously onto seeds and severely retarded germination and seedling growth. In comparison to HCl, a considerably lower dose of HF gas reduced seed growth.

PSG and seedling development were not affected when soil replaced filter paper in exposures of seeds to HCl. Similar soil tests were conducted with HF as the toxicant. After exposing tomato seeds or cups of greenhouse soil mix to 9.5 mg HF m⁻³ for 20 minutes, rinsing some seeds, and placing seeds on soil surfaces, the seeds were incubated in the dark for 23 days. HF gas reduced PSG and seedling length of seeds on soil (Table 43). Poorest seedling development (90% length reduction) occurred when seeds were exposed on filter paper then transferred to unexposed soil. Growth of exposed rinsed

TABLE 41
DEVELOPMENT OF TOMATO SEEDS ON FILTER PAPER AFTER EXPOSURE OF
SEEDS OR FILTER PAPER TO HF GAS FOR 20 MINUTES

Treatments		Germination ¹ (%)	Length (cm)	
Seeds	Paper		Epicotyl	Hypocotyl
HF	HF	0	0.0	0.0
No gas	HF	0	0.0	0.0
HF	No gas	50	3.8	1.1
No gas	No gas	97	5.2	1.9

¹Mean of three replicates, 20 seeds each

TABLE 42

DEVELOPMENT OF TOMATO SEEDS ON FILTER PAPER AFTER EXPOSURE OF SEEDS OR
FILTER PAPER TO HF GAS, WITH UNEXPOSED AND RINSED CONTROLS

Treatments		Germination ¹ (%)	Length (mm)	Length Reduction (%)
Seeds	Filter Paper			
HF	HF	0	0.0	100
No gas	HF	0	0.0	100
HF	No gas	2	3.0	92
HF then rinsed	No gas	88	21.6**	44
No gas	No gas	100	38.5**	0

¹Mean of three replicates, 20 seeds each

**Differences between marked means significant at 1% level

TABLE 43

DEVELOPMENT OF TOMATO SEEDS EXPOSED TO HF GAS AND INCUBATED ON SOIL
ALSO EXPOSED TO HF, WITH UNEXPOSED AND RINSED CONTROLS

Treatments		Germination ¹ (%)	Length (mm)		Length Reduction (%)
Seeds	Soil				
HF	HF	75	9.2 ²	b	46
No gas	HF	68	5.8	b	66
HF	No gas	45	1.7	a	90
HF then rinsed	No gas	63	8.8	b	48
No gas	No gas	97	17.0	c	0

¹Mean of three replicates, 20 seeds each

²Means followed by the same letter are not significantly different at 5% level by Duncan's Multiple Range Test

seeds was reduced to 48% of control seedling lengths. Soil exposed to HF apparently retained enough toxicant to inhibit seed development.

Effect of Age on Plant Sensitivity

Pinto beans of 8, 12, 16, 20, 25, or 28 days of age were exposed for 20 minutes to determine whether age affects plant sensitivity to HF gas. Three plants of each age were treated to one of two concentrations and treatments were repeated on two dates. Injury on plants exposed to 2.0 mg HF m⁻³ was

statistically lower ($F = 25.2$, $p = 1\%$) than injury on plants exposed to 3.4 mg HF m^{-3} (Figure 14). Primary bean leaves were most sensitive to HF gas when 16 to 20 days old. Primary leaves on younger and older plants were less susceptible. The first set of trifoliate leaves were sensitive to HF as they expanded.

Fluoride in Leaves of Plants Exposed in Age Trials

Bean leaves from plants exposed on the same day but in different chambers, were harvested 48 hours after exposure. Some leaves were rinsed, then all were thoroughly dried and finely ground. Fluoride was released from tissue by combustion in an oxygen atmosphere, absorbed in buffer, and measured. Fluoride content was calculated by weight (Table 44).

Fluoride content of plants exposed to one concentration (3.5 mg HF m^{-3}) was compared to those exposed to a lower level of HF (2.2 mg m^{-3}). A t-test was used to compare visual injury and fluoride content with HF concentrations including plant age, plant parts, and rinsing methods. Leaves exposed to 3.5 mg HF m^{-3} contained significantly more fluoride ($t = 3.14$, $df = 19$, $p = 1\%$) than tissue from plants exposed to 2.2 mg m^{-3} . Rinsing leaves after harvesting did not alter their fluoride content. The mean fluoride content of rinsed and unwashed leaves was not different ($t = 0.30$, $df = 15$, N.S.).

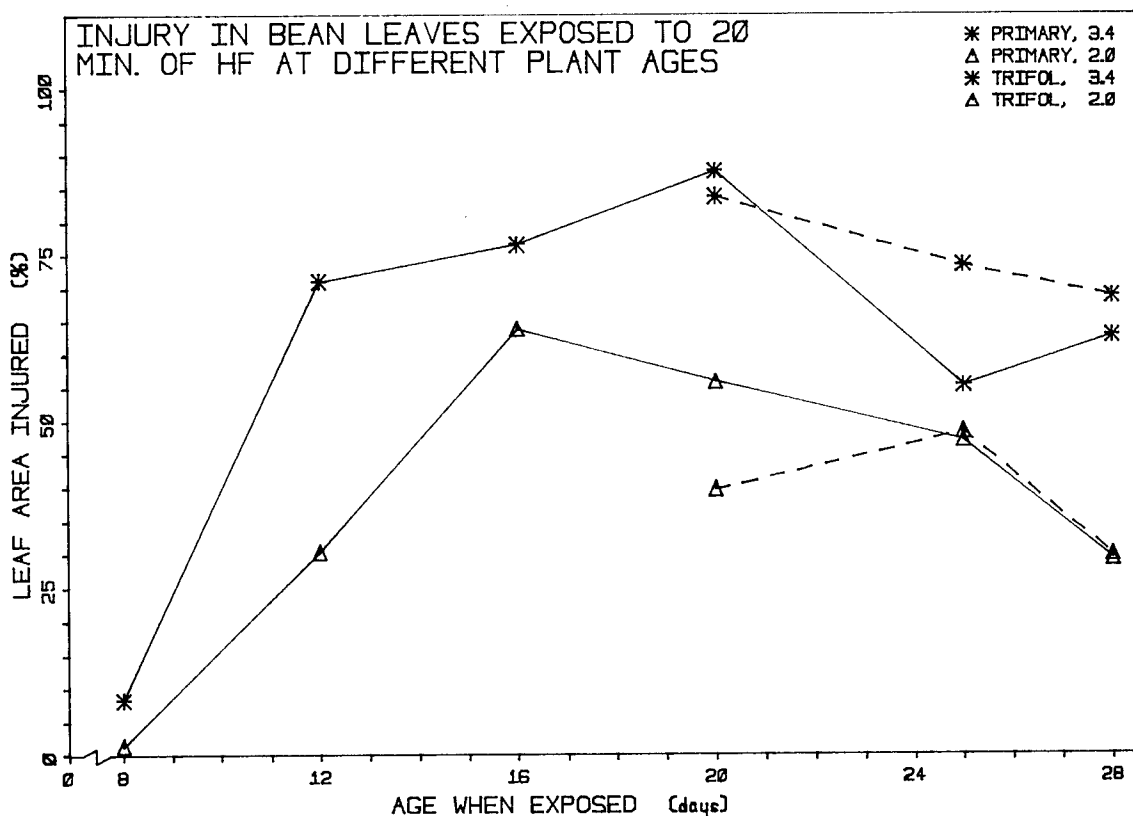


Figure 14. Injury on primary (solid lines) and trifoliate (dashed lines) leaves of pinto beans of different ages exposed to 20 minutes of HF gas at two concentrations.

TABLE 44
FLUORIDE CONTENT OF LEAVES FROM BEAN PLANTS
EXPOSED TO HF GAS FOR 20 MINUTES

Plant Age (days)	No. Plants	HF Conc. (mg m ⁻³)	Leaf Rinsed (+) or Unwashed (-)	Leaf Sampled	Leaf Area Injured (%)	Tissue F (μg F g ⁻¹)
8	3	3.5	+	Primary	27.1	594
8	3	2.2	+	Primary	4.2	414
12	3	3.5	+	Primary	66.7	1086
12	3	2.2	+	Primary	12.5	985
16	3	3.5	+	Primary	83.4	1282
16	3	3.5	+	1st Trifoliate	0.0	390
16	3	2.2	+	Primary	72.9	834
16	3	2.2	+	1st Trifoliate	0.0	362
20	1	3.5	-	Primary	87.5	822
20	1	3.5	-	1st Trifoliate	75.0	1330
20	2	3.5	+	Primary	87.5	862
20	2	3.5	+	1st Trifoliate	81.2	1382
20	1	2.2	-	Primary	81.3	466
20	1	2.2	-	1st Trifoliate	12.5	734
20	2	2.2	+	Primary	59.4	678
20	2	2.2	+	1st Trifoliate	50.0	958
25	1	3.5	-	Primary	25.0	390
25	1	3.5	-	1st Trifoliate	87.5	726
25	1	3.5	-	2nd Trifoliate	87.5	742
25	2	3.5	+	Primary	75.0	614
25	2	3.5	+	1st Trifoliate	81.2	998
25	2	3.5	+	2nd Trifoliate	81.2	802
25	1	2.2	-	Primary	12.5-s	778
25	1	2.2	-	1st Trifoliate	50.0	910
25	1	2.2	-	2nd Trifoliate	50.0	778
25	2	2.2	+	Primary	6.2-s	358
25	2	2.2	+	1st Trifoliate	81.2	814
25	2	2.2	+	2nd Trifoliate	81.2	718
25	2	2.2	+	3rd Trifoliate	62.5	762
25	2	2.2	+	4th Trifoliate	0.0	354
28	1	3.5	-	Primary	62.5-s	670
28	1	3.5	-	1st Trifoliate	87.5	802
28	1	3.5	-	2nd Trifoliate	87.5	646
28	2	3.5	+	Primary	75.0-s	598
28	2	3.5	+	1st Trifoliate	87.5	586
28	2	3.5	+	2nd Trifoliate	87.5	574
28	1	2.2	-	Primary	50.0-s	558
28	1	2.2	-	1st Trifoliate	37.5	270
28	1	2.2	-	2nd Trifoliate	75.0	550
28	2	2.2	+	Primary	34.4-s	454
28	2	2.2	+	1st Trifoliate	31.2	486
28	2	2.2	+	2nd Trifoliate	31.2	514
28	2	2.2	+	3rd Trifoliate	18.8	734

Results with plants in these tests confirmed earlier conclusions: leaves from plants exposed to 3.5 mg HF m^{-3} were injured more than those exposed to 2.2 mg m^{-3} . This was true whether primary leaves ($t = 4.13$, $df = 8$, $p = 1\%$) or both primary and trifoliate leaves ($t = 5.17$, $df = 15$, $p < 0.1\%$) were considered. Injury on trifoliates correlated linearly with plant age ($r = 0.69$, $F = 11.06$, $p = 0.05\%$). Fully developed primary leaves were most sensitive to HF-induced injury with younger expanding and older senescing leaves resisting injury.

Fluoride content was dependent on plant age at time of exposure to HF gas. Old and very young leaves contained less fluoride than fully expanded leaves (Figure 15). Fluoride uptake was greatest in leaves younger than those that were injured most (Figure 16). Sensitive leaves which become severely injured may not be capable of further fluoride uptake due to extensive necrosis.

Conclusions on Exposing Plants to Short Periods of HF Gas

Very few air pollution investigations consider effects of short exposures on plants. HF, highly toxic in small concentrations, was here shown to damage plants exposed for 20 minutes at sufficiently large gas concentrations. Initial plant responses during short exposure periods probably involved

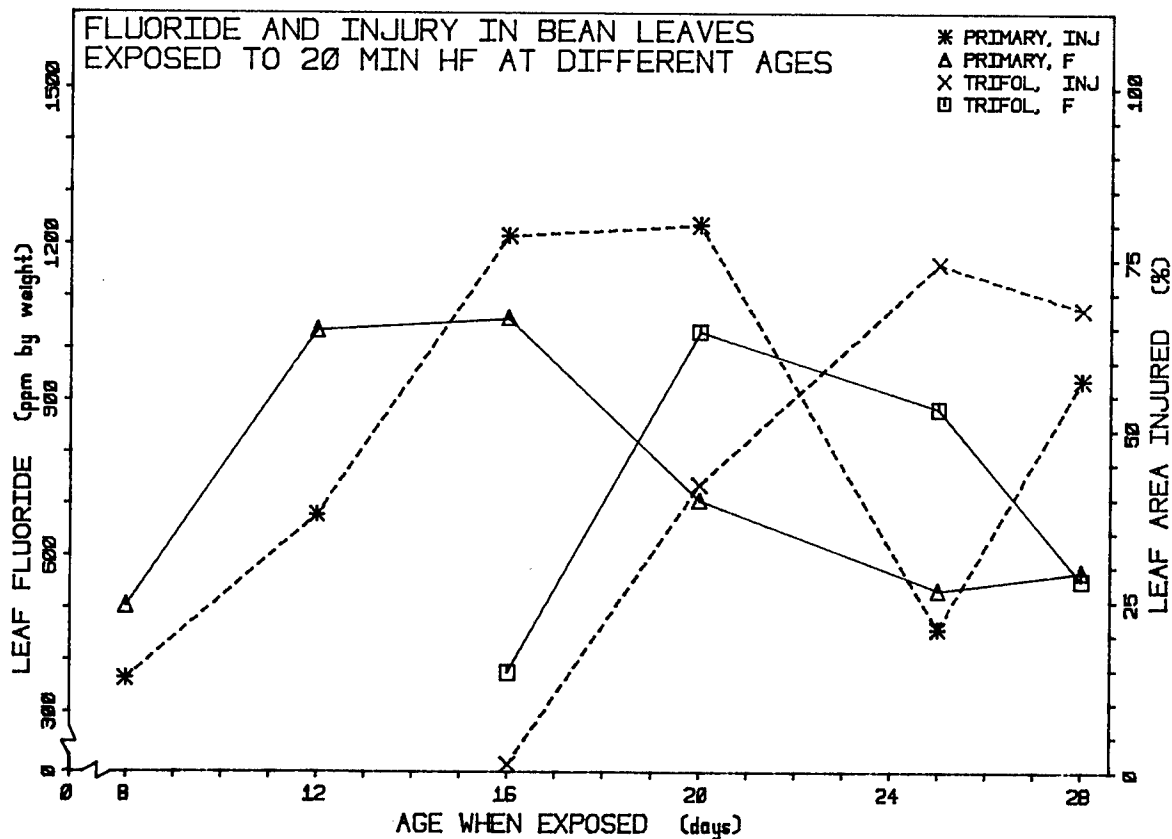


Figure 15. Fluoride content of leaves of 8- to 28-day-old bean plants exposed to 3.5 or 2.2 mg HF m^{-3} for 20 minutes.

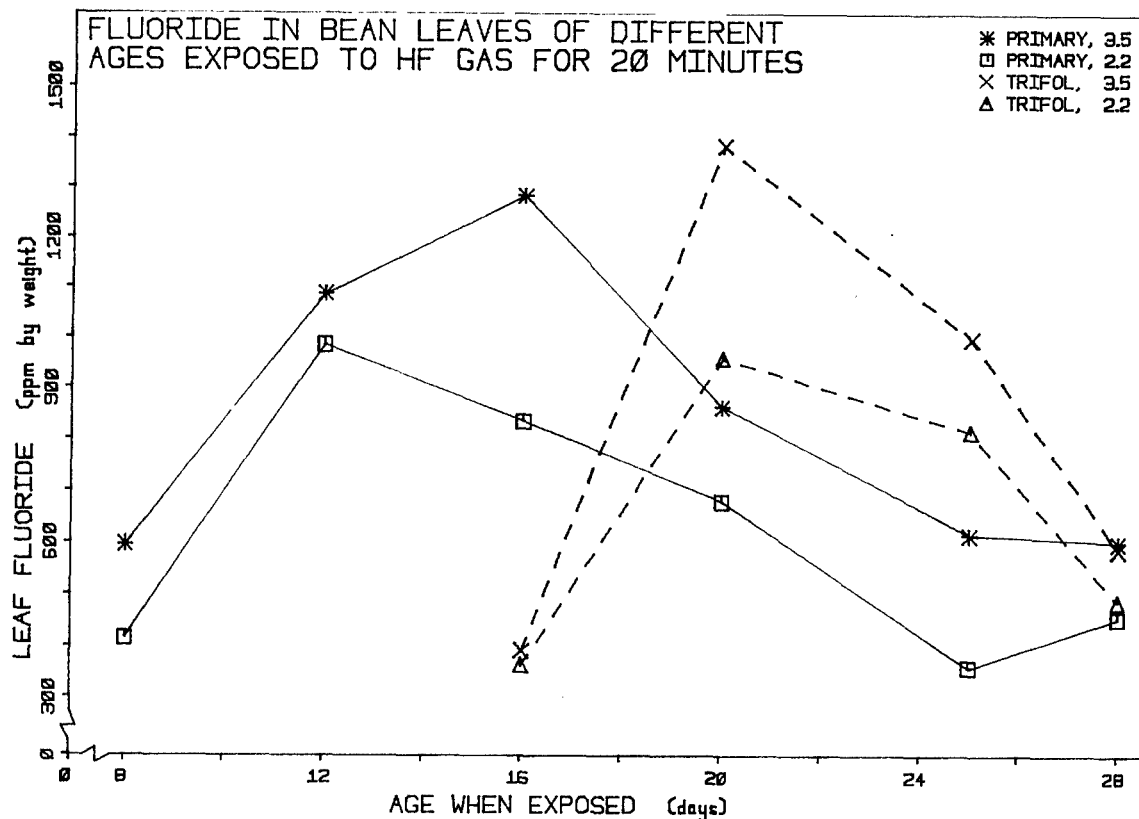


Figure 16. Fluoride content (solid line) and injury (dashed line) of leaves from 8- to 28-day-old bean plants exposed to ca 2.7 mg HF m⁻³ for 20 minutes.

massive membrane and tissue destruction rather than active uptake and physiological reactions. Plants have different thresholds, with injury symptoms visible on sensitive species at smaller gas concentrations than on resistant species. Active, mature leaves were most susceptible to HF gas. Measurable foliar uptake of fluoride occurred during short exposures to HF. Since rinsing leaves did not reduce the amount of retrievable fluoride, significant uptake took place across physical leaf barriers. Exceptionally strong surface forces may have bound or adsorbed the fluoride. Further studies should determine whether foliar fluoride can be translocated within the plant.

DISCUSSION

This report completes a five-year study on the effects on vegetation of certain air pollutants associated with solid fuel rocket motor exhausts. The results have been detailed in annual reports, journal papers, and conference presentations. The types of pollutants and the conditions for the plant exposures were somewhat unique. HCl and HF, strong corrosive gases, rarely are found in the atmosphere at the concentrations investigated here, but might be present in exhaust ground clouds following a rocket launch. Since a

rocket ground cloud would probably dissipate rapidly, we limited our exposures to 20 minutes; in contrast, most other pollution episodes are measured in hours or days. We also dealt with the phytotoxicity of aluminum oxide (Al_2O_3) particles and HCl mist, other exhaust cloud constituents. To conduct these investigations, we developed generating systems, constructed exposure chambers, and devised detection systems for the pollutants and grading systems for the plants.

Aluminum oxide was non-toxic as a single pollutant and the phytotoxicity of HCl plus Al_2O_3 was traced to the HCl component. HF was considerably more injurious to plants or seeds than HCl.

The majority of our investigations concerned gaseous HCl. Plant injury was characterized as glazing or burning. Phytotoxicity was dependent on many factors besides HCl dose: variety, age, condition of and nutrient availability to the plant, and exposure conditions such as light, temperature, and relative humidity. Sensitivity to HCl was not altered by infecting plants with pathogenic viruses or beneficial mycorrhizae or by applying an antioxidant material. Increased injury occurred when the insecticide Cygon was applied, when high humidity or dew was present, or when plants were exposed repeatedly to HCl. Resistance to injury was greater in field-grown than greenhouse plants, in plants grown under elevated salt (NaCl) regimes, and in older plants and woodier species.

Our work continues nearly 30 years of investigations into the effects of air pollution on plants. Most earlier work differed from ours in that the pollutants others studied were often ozone, sulfur dioxide, or peroxyacetyl-nitrate, and the doses were small concentrations, in the ppm or ppb range, lasting for long periods, from hours to days or weeks. Long-term studies with low levels of HCl or HF indicated plant uptake and translocation of the pollutant affected subsequent physiological changes (Guderian, 1977; Jacobson et al., 1966). Injury seemed to be restricted to a surface phenomenon when plants were exposed to large HCl concentrations for relatively short periods (Endress et al. 1978).

Some investigators have dealt with the phytotoxicity of short exposures of HCl, HF, and rocket fuel exhausts. Heck et al. (1962) exposed plants, soil, and aquatic animals to 21 missile fuel components in liquid and gaseous form. They found that symptoms developed after 13 minutes on plants exposed to 62 ppm gaseous chloride. MacLean et al. (1968) reported citrus defoliation after exposure to 0.4 mg HF m^{-3} for 30 minutes. Nimmo et al. (1974a,b) exposed plants for 30 minutes to burning solid rocket fuel. In laboratory studies, growth was reduced in citrus, peas, and bush beans at concentrations of 6 to $760 \text{ mg HCl m}^{-3}$, while field studies indicated no structural damage at 8 to 152 mg m^{-3} (Nimmo et al., 1974b). In recent years, Endress and co-workers have explored the microscopic and physiological aspects of plants exposed to HCl at phytotoxic levels for 20 minutes. They found abaxial glazing more prevalent than bifacial necrosis after exposure to 6 to 509 mg m^{-3} (Endress et al. 1978). Glazing was associated with the collapse of the epidermal cells seen with the light microscope. Surface injury phenomena were not dependent on gas entering stomata. Single HCl treatments reduced the rate of leaf expansion (Endress, Oshima, and Taylor, 1979). Membrane disruption, chloroplast deformation, and crystal development were found on the ultrastructural level; these subcellular alterations were reversible depending

on leaf age, time of sampling after treatment, and HCl concentration (Endress, Kitasako, and Taylor 1979b; Heath and Endress, 1979).

Heck et al. (1980) recently reported on the ecological effects of HCl, Al_2O_3 , and rocket fuel exhausts on Florida plant species. Their results were similar to ours. Twenty-four native species were screened. Radish and soybeans, with injury threshold concentrations of 14 and 24 mg HCl m^{-3} for 10 minute exposures, respectively, were the most sensitive cultivated plants tested. Heck's group found greater sensitivity in plants exposed in fall and spring than in winter and in plants exposed under increased relative humidity or when leaves were wet. Chloride in exposed soybean leaves correlated positively with dose but moved little within the plant. Al_2O_3 was not phytotoxic in their tests.

In conclusion, the rocket exhaust products we studied were gaseous HCl or HF and Al_2O_3 particles. Of these, Al_2O_3 was non-toxic whereas HCl and HF were potential phytotoxicants under greenhouse conditions at 8 mg m^{-3} and 3 mg m^{-3} , respectively. Single 20-minute exposures at these concentrations produced injury from which the plant generally recovered. Repeated episodes, however, reduced plant growth, yield, and biomass. Plants were more sensitive if exposed during midday. Field-grown plants were generally more resistant than greenhouse plants.

It would probably be difficult to find evidence of any plant injury symptoms from occasional rocket exhaust products impacting native species or field crops. It is less certain, however, that repeated exposures to the exhaust clouds would be harmless to plants, even if the toxic gas concentrations in those clouds are as low as predicted by current Air Force models. The possibility of plant effects caused by HCl- or HF-laden precipitation following rocket launches has yet to be adequately explored.

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